33rd WEFTA Meeting
Reykjavik – Iceland 2003

48th Atlantic Fisheries Technology Conference

Atlantic Fisheries
Technology Conference

TAFT 2003

FIRST JOINT TRANS ATLANTIC FISHERIES TECHNOLOGY CONFERENCE

10–14 June 2003 Reykjavik, Iceland

Proceedings of the TAFT 2003 conference
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Time Schedule TAFT 2003 June 10-14 Time	Tuesday	Wednesday	Thursday		Friday	Saturday	Time Schedule TAFT 2003 June 10-14 Time
		Plenary session	Parallel session I	Parallel session II	Plenary session	Plenary session	
08:00		Registration					08:00
08:30		Welcome and Introduction Funding possibilities	Quality	Traceability	Workshop Novel components and utilization of by-products		08:30
09:00						Aquaculture	09:00
10:00 - 10:15							10:00 - 10:15
10:45		Networking in Fisheries research Poster Session					10:45
11:30			Lunch			Closing	11:30
12:00 - 13:00		Lunch	Parallel session I	Parallel session II	Lunch		12:00 - 13:00
13:00		Parallel session I: Innovative handling and processing Parallel session II: Fish products for health	Safety	Functional properties of fish components	Novel components and utilization of by-products Panel discussion	Lunch Excursion - Reykjanes peninsula and the Blue Lagoon	13:00
15:00		Parallel session II: Consumer perceptions					15:00
16:00	Registration						16:00
17:00	WEFTA directors meeting and AFTC Executive Committe meeting		Networking				17:00
19:00	Joint WEFTA/AFTC meeting	Reception at City Hall	Viking Fest		Gala Dinner Perlan		19:00

FOREWORD WEFTA / AFTC

It is a great pleasure to witness the first Trans-Atlantic Fisheries Technology Conference becoming a reality. It is a joint effort of two independent organisations, the Atlantic Fisheries Technology Conference (AFTC), based in North America, and the West European Fish Technologists' Association (WEFTA), based in Europe. Each organisation has organised annual meetings on their respective continents, but discussing similar topics of relevance for the fish processing industry. Invitations for participating in each other's meetings have been communicated from both sides, and some members have used this opportunity to cross the Atlantic for attending meetings and workshops not being on their home continent. New contacts and friendships have resulted. Communication over large distances have become better and faster with the electronic development making Internet and e-mail exchange a convenient way of talking together. However, it is still necessary to meet in order to develop human relationships, to exchange views in open discussions, and to enjoy and sense the atmosphere during the social part of the meeting arrangements. This can never be substituted, even by using the excellent tool of videoconferences. So it must be right to arrange meetings between the continents to take full advantage of the development where the world gets smaller and we get closer every day. After good meetings it is easier to fully utilize the potentials of electronic communications between the meetings, so we are on the right track. The TAFT meeting represents the beginning of a new era for collaboration over large distances with common goals of promoting good fish technology. The Icelandic Fisheries Laboratories are sincerely thanked for taking on the duty of arranging this conference as a fine representative of WEFTA. I wish you all a successful conference, and may it become the nucleus for a fruitful network for all of us.

Torger Børresen
Scientific Program Committee representative
West European Fisheries Technologists' Association

The importance of the first Trans-Atlantic Fisheries Technology Conference cannot be overstated. Every year a few hearty souls from our two regional fisheries associations would venture out and participate in association meetings outside their own region. Today, we are witness to what generations of venturous individuals thought about but never dared, a joint conference of the Western European Fish Technologists' Association and the Atlantic Fisheries Technology Conference. Now after more than thirty years, one question comes to mind. "What took us so long?" With globalization of trade and reduction in distance through technology of the Internet and computers, the only limit we face is time itself. As you prepare for this year's joint conference, let us renew our commitment to "encourage and mentor young scientists, educators and policy makers" in fulfilling our goal of "sustainable fisheries to ensure a high quality of life." On behalf of the Atlantic Fisheries Technology Conference, I offer my congratulations to each TAFT conference attendee. Let us pledge not to allow such a long period of time to pass before our next joint conference. Best wishes for a meaningful conference and sincere gratitude to our hosts, the Icelandic Fisheries Laboratories and WEFTA. May your nets always be full and your hearts warm.

David Green
Scientific Program Committee representative
Atlantic Fisheries Technology Conference

WELCOME ADDRESS

In the past decade major developments have taken place in fisheries throughout the world, both with regard to fishing and fish processing. Catches have increased tremendously, to the point where many of the fishing stocks in the world's oceans are fully exploited or even over-exploited.

The main task facing us now as far as fishing is concerned is to manage fisheries in such a way as to optimally utilise the capacity of individual stocks and the ecosystem as a whole.

To add value and improve the utilisation of the valuable and unique re-source which fishing stocks comprise, more attention must be paid to how the raw material is utilised. More emphasis needs to be placed on research and development in food production from marine products and on other products which can be produced from raw material which currently is poorly utilised or even regarded as waste.

It is encouraging to learn that associations of research institutes in the area of marine research on both sides of the Atlantic, WEFTA and AFTC, have decided to hold their first joint meeting in Iceland in 2003. Participation in WEFTA has been important for Icelandic research in this area.

Co-operation between WEFTA and AFTC should lead to further strengthening R&D work in both Europe and America, through exchange of information or co-operation between institutes on projects. I have no doubt that this conference will give further impetus to the activities of the associations in general and between individual institutions within them.

The new opportunities thus opening up give us exciting new possibilities.

Árni M. Mathiesen, Minister of Fisheries

ADDRESS FROM THE IFL

It is a great honour for Iceland and the IFL to host the first joint trans-Atlantic Fisheries Technology Conference on behalf of WEFTA and the AFTC. I hope the conference will be remembered later on as having been a success, both with regards to the issues which will be discussed here as well as to the personal relationships and networking that will be established in Reykjavík.

We who are gathered here in Reykjavík in 2003 for the TAFT conference and who are in the business of carrying out research and giving the fisheries industries advice on the subject of fish technology, fish processing etc. are perhaps confronted with a greater task than ever before.

A recent letter, published in the journal Nature, and later discussed in an editorial in The New York Times, presented a rather gloomy picture of the current state of the world's oceans, which, according to the report "are in trouble, its coastal waters increasingly polluted and its fish stocks in various stages of decline." The report maintains that the world's biggest and most economically important species of fish, including cod, halibut, tuna and swordfish have been decimated over the last 50 years.

If the two Canadian scientists, who wrote the letter in The Nature, are right in their presumptions, it goes without saying that this is a problem that obviously requires global, definitely painful solutions which will demand political courage and money, as the editors of TNYT point out. But it also means that in the future we should increasingly focus both on how to make more out of less and on aquaculture. Utilising as much of and adding value to the total allowable catches (TACs) will be one of the most important tasks of the fishery industry worldwide in the 21st century. And that is precisely what makes us, as scientists specialising in the subject of fish processing technology, more important than ever before.

The topics of the TAFT 2003 conference reflect the current emphasis in the area of fish technology research, i.e. increased value from seafood and how to ensure the safety of seafood for the consumer. The focus is on consumer driven development of tailor-made, functional seafood products to improve health and to ensure nutritional quality and safety by full utilisation of raw materials from aquaculture and from traditional fisheries.

This is in concordance with increasing awareness of consumers about the safety aspects of food products in general and health promotion. International networking in fisheries research is crucial to ensure the development of good quality, health promoting, safe fish products for consumers. This conference is a great opportunity for the scientists and the industry in Europe and North-America to meet and hopefully it will be a milestone in a fruitful cooperation in this area between these two continents in the future.

Sjöfn Sigurgísladóttir, director IFL

NETWORKING IN FISHERIES RESEARCH

A Network in Fisheries Research will be launched in connection with the TAFT 2003 conference to stimulate international research on the development of healthy and safe fish products. The Nordic Industry Fund and the Icelandic Centre for Research are sponsors of the networking event

Aims

- to establish a network to promote international cooperation in fisheries research.
- to explore possibilities for cooperative projects.
- to stimulate the development of international fisheries research projects.

Procedure

1) Participant have filled out a questionnaire on their interest in research topics and networking groups have been formed.
2) The conference itself is a forum for presenting current projects. Discussion in both the oral and the poster sessions will give additional ideas for networking topics.
3) Networking meetings will be held and project ideas discussed.
4) Reports from networking meetings and session reports from moderators will be posted on the website: http://www.rf.is/TAFT2003, giving immediate information on the results from the networking event.

Funding

Representatives from the European Commission and the funding agencies in USA and Canada will present an overview on funding possibilities for joint projects.

Outcome

The outcome of this event will become a basis for new cooperative research and development projects in fisheries and food research with the participation of scientists as well as representa from the industry on both sides of the Atlantic.

The overview of funding possibilities gained at the conference will stimulate and make common projects a reality in the near future.

<http://www.rf.is/TAFT2003>www.rf.is/TAFT2003

All material and information related to the networking at the conference will be posted on the website of IFL www.rf.is/TAFT2003

RANNÍS

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L 1. REVIEW OF EUROPEAN UNION RESEARCH FUNDING FOR FISHERIES TECHNOLOGY DURING THE FAIR (FP4) AND QUALITY OF LIFE (FP5) FRAMEWORK RESEARCH PROGRAMMES

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Abstract

The start of the sixth framework programme of the European Union is a very good moment to review the types of projects that received funding under the two previous programmes. When establishing what research topics have been covered by the projects involved, a first analysis of research impacts can be established. Also, by comparing statistics within and among different scientific domains an estimate of future areas in need of research as well as a first glimpse of the potential societal impact may be estimated.

The FAIR programme was a sub-programme of FP4, which ran from 1994 until 1998 resulted in a number of seafood and technology related projects that motivated researchers to co-operate in research projects throughout Europe. Research alliances were created and methodologies compared between research centre laboratories, universities and industries. The Quality of Life programme was a sub-programme within FP5, and created further funding possibilities for fisheries technologies from 1998 until 2002. Between these two programmes a shift in operational responsibilities resulted in transfer of seafood technology funding from DG Fish to DG Research. The fisheries technology researchers had to compete with agriculture, nutrition and general food science technologies. The funding analysis shows a change in success for proposals in this area securing funding, both in number of proposals and amount of funding for fisheries technologies. Consecutively the sixth framework programme has gone one step further in amalgamating all the different food science domains into a single research arena. In this programme aquaculture research has been added to the portfolio in Thematic Priority 5 " Food Quality and Safety". This can be looked at as a new challenge and opportunity for fisheries/food technology researchers, and requires a solid analysis of the past funding of topics to create a pragmatic view towards the future.

The present paper establishes some of the very basic comparative results of the scientific domains covered. The aim is to provide a first overview of scientific research funding and topic coverage in fisheries and food technology over the last eight years. Furthermore, an attempt will be made to look towards the future, on basis of an estimation of the potential impacts achieved by the research activities in this field over the concerned period.

Introduction

The funding regimes of the EU Community research programmes have been evolving and changing with time. Each Framework Programme (FP) has had its own set of goals and criteria that link to the overall development of the European Union, aiming to strengthen the human potential within the Community. The table to the right shows an overview of the relevant sub-programmes dealing with the research in the food related sectors for FP4, FP5 and FP6.

In FP4 and FP5, this sectarian approach of the industries is being clearly reflected, with AGRI; FISH; and FOOD, nutrition and health research operating within different areas or key actions. The research clients knew where to apply for funding, based on their respective food sectors. However one significant shift from this main theme occurred between FP4 and FP5, where seafood research was moved from the FISH sector over to the FOOD sector under KA1 of the quality of Life sub-programme. At the same time AGRI research funding was transferred from Directorate General (DG) Agriculture to DG Research. DG Fisheries maintained their research unit, but from the beginning of FP5, concentrated on Fisheries and aquaculture research.

A third, and probably final, shift in food research management at the EU level happened when FP6 was launched in 2002. All food research has been concentrated at DG Research in thematic Priority 5 "Food quality and safety". This includes agriculture, fish, seafood and aquaculture, as well as general food science, nutrition, health and food safety research. In FP6 DG Fisheries maintains research in fisheries and aquaculture in the field of Scientific support to Policies (SSP) as this may be applicable to policy development, and is mainly concentrating on fishery management, environmental and aquaculture policy related issues. In writing the work programmes for the different calls for proposals it is ensured that no duplication of efforts with other thematic priorities takes place.

[1] "Disclaimer: The views expressed in this document are purely those of its author and may not in any circumstances be regarded as stating an official position of the European Commission"

The thrust of FP6 is to create a more competitive Europe by creating a European Research Area by focusing research on more strategically competitive areas. The emphasis is therefore on larger projects and ambitious joint mobilisation of resources. New funding instruments have been introduced, in which it is anticipated that grants to the budget can be on the scale of tens of millions of Euro. The shift can be foreseen that funding will move from project funding to a pan European programme funding of selected core themes.

FP4 - Agriculture and Fisheries (FAIR)		
6 Areas of research	***M€***	***%***
1 - Integrated production & proc. chains	111	15%
2 - Scaling-up & processing methodol.	52	7%
3 - Generic sci. & tech. for nutritious foods	118	16%
4 - Agriculture, forrestry & rural developm.	274	37%
5 - Fisheries & Aquaculture	126	17%
6 - Objectives addressed by concertation	59	8%
Total	**740**	**100%**

FP5 - Quality of Life, 6 Key Actions	***M€***	***%***
1 - Food, nutrition and health	290	12%
2 - Control of infectious diseases	300	12%
3 - The "cell factory"	400	17%
4 - Environment and health	160	7%
5 - Sustainable agriculture, fisheries	520	22%
6 - Aging population	190	8%
Subtotal *(77% of total)*	*1860*	
RTD activities of a generic nature	483	20%
Support for research infrastructures	70	3%
Total	**2413**	**100%**

FP6 - Priority 5 and SSP	***M€***	***%***
P5 Food quality and safety	685	84%
Scientific Support to Policies		
Agri & foods	74	9%
Fisheries and aquaculture	55	7%
Total	**814**	**100%**

Discussion

The impact of EU Community funding of research projects has resulted in creating a new fabric of science within Europe over the last 12 years. The long term impact of funding thousands of student grants through the EU research programmes will be measured by the success stories that have come to light, and others that will be surfacing in years to come. The research within the food sectors has changed from being mono-national to multi-national, which was the main impact of the FP2 or FLAIR programme. The FP3 (AIR) resulted in consolidated scientific teams and shifted from being mono-disciplinary towards multi-disciplinary. In FP4 (FAIR) the science teams became larger, transfer of know-how gained increased importance, and the multi-disciplinary project actions were further consolidated. In FP5 the projects increased in size and more funding became available per partner. The co-operation of different disciplines, crossing borders and regions have shortened both physical and cultural distances.

The concept of an integrated multi-disciplinary approach to projects was further developed in FP5 by clustering a number of projects together for increased impact. This will be taken further by the introduction of the large Integrated Projects and Networks of Excellence in FP6.

By analysing the food sector research in FP4 and FP5 it can be observed AGRI research is stable and is by far the largest segment, but this includes forestry and some non-food related research. Food and nutrition research funding has increased between the two programmes, from 23% to 32% respectively. Seafood seems to be loosing out as it represented approximately 5% in FP4, but only 3% of the FP5 food sector research actions. Table 1 shows the split between food sectors for the two programmes, based on number of RTD and CRAFT/SME (Small and Medium Enterprise) projects.

In FP4 the average research project funding was about 0,8 million Euro (M€) with 7 to 8 partners. For CRAFT projects the average project funding was 0,4 M€. The funding per project increased to about 1,5 M€ in FP5 with a similar number of partners in the teams, and funding for CRAFT projects increased to an average of 0,5 M€.

It is not simple to measure the impact of the research projects as the intangible value may by far be the more important element, and to accurately evaluate this may be near to impossible. Statements have been made by some stakeholders that the EU research programmes may prove to be the critical adhesive for the future of the European Union with at least 25 different nations involved. The partnerships struck within the projects may result in numerous future business ventures and/or opportunities that cross national borders, thereby creating further impacts in the years to come.

Table 1. Percentage of research projects by domains

Research Domains	***FP4 FAIR***	***FP5 QoL***
Agriculture	46%	45%
Food & nutrition	23%	32%
Seafood	5%	3%
Aquaculture	17%	10%
Fisheries	9%	10%
Total	100%	100%

As an example of measurable impacts, a recent survey of 86 finished FP4 (FAIR) fish projects showed the following:

- Teams involved 1923 persons of which 1113 were scientists,
- 589 scientific publications in refereed journals, and additional 269 submitted papers,
- 287 students were trained resulting in 120 dissertation thesis
- more than 245 technicians were trained
- about 44% of the project teams stated that the work had delivered inputs into policy making or for regulations / legislation.

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L 2. ENSURING GLOBAL COMPETITIVENESS OF THE U.S. SEAFOOD INDUSTRY – A NATIONAL SEA GRANT INITIATIVE

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Abstract

The National Sea Grant College Program is a federal/state partnership funded through the U.S. Department of Commerce's National Oceanic and Atmospheric Administration and 29 state governments. The national program promotes the wise use of coastal and marine resources through research, extension and education. Ten theme teams recently were formed to focus on national issues and needs in specific areas of expertise including seafood-processing technology. The U.S. seafood industry faces many challenges and opportunities as it enters the 21st century. The Seafood Science and Technology Theme Team has identified a number of issues and needs for ensuring the global competitiveness of the U.S. seafood industry. This presentation will summarize the findings of the group. In this new seafood era, science and education have become cornerstones for maintaining the vitality of the nation's $27 billion dollar seafood industry and its 250,000 workers. Numerous research and technology transfer needs identified in the report could help invigorate the U.S. seafood industry. Sea Grant has more than 30 years of experience working in nearly every state and involving every type of seafood product – a proven track record of collaboration by university research and extension personnel with businesses, government, research laboratories and consumers.

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L 3. FUNDING POSSIBILITIES FOR NETWORKING RTD PROJECTS

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Abstract

The Nordic Industrial Fund - *centre for innovation and commercial development* is an institution under the Nordic Council of Ministers.
Our aim is to strengthen the Nordic business sector through the creation of a Nordic knowledge market. We do this by initiating and financing projects and activities that create synergy between the actors in the Nordic innovation system. Our mandate - the Fund's political task - is to strengthen the Nordic business sector, which in more concrete terms means increasing the competitiveness of Nordic industry, reinforcing Nordic business culture and securing sustainable development in Nordic society. Our vision – Nordic area as a knowledge market-implies that the projects and other activities financed by the Fund will help to enable the commercial and public sectors to find the knowledge and expertise they require in the Nordic area. Ideally, an effective Nordic knowledge market will encourage non-Nordic specialist companies to move their operations here, while professional opportunities open to highly educated Nordic residents will encourage them to remain living and working in Nordic area.

Our strategy - the synergy model - is based on our work in areas of importance to the Nordic knowledge market and the Nordic business sector. Through these priority areas, the Fund shall work to create synergy between the actors in the Nordic innovation system.

RANNÍS, - The Icelandic Center for Research, is established by a new legislation enacted in 2003 and replaces the office of the earlier Icelandic Research Council established by legislation in 1994. This in turn replaced earlier councils that trace their origins to a research council structure set up before the Second World War.

RANNÍS reports to the Ministry of Education, Science and Culture and its mission is to provide professional assistance to the preparation and implementation of science and technology policy in Iceland.

RANNÍS is responsible for operating the financial support system for research and technical development under the the Council for Science and Technology Policy and provides services to its subcommittees and the Advisory Boards and the Boards of Directors for the main funds. This includes the **Research Fund**, the **Instruments Fund** and the **Graduate Education Fund** under the Ministry of Education, and the **Technology Development Fund** under the Ministry of Industry. Also RANNIS Coordinates and promotes Icelandic participation in international cooperation in science and technology and interacts with corresponding agencies in other countries. The paper discusses possibilities in funding of international co-operation RTD projects.

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L 4. FISH SAFETY AND QUALITY IN TIMES OF GLOBALIZATION

Grimur Valdimarsson, Roland Cormier, Lahsen Ababouch
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Summary

Fish and fishery products are the most internationally traded foodstuffs in the world with about 37% (live weight) being sold across national borders. In value terms over 50% of this trade originates in developing countries. The globalization of fish trade has increased the risk of cross-border transmission of hazardous agents. This is illustrated by recent detentions and rejections by the European Union of imported shipments of fish and fishery products due to chemical and drug residues. The rapid development of aquaculture has indeed opened a new dimension regarding fish safety that will demand more attention in the near future. Thus the need for an international approach to minimize the risk posed by fisheries products to consumers has become increasingly clear. The SPS and TBT agreements (1995) of the World Trade Organization are all important in this regard. The food and feed scares of recent decades as well as developments in food processing to meet consumers' quest for minimally processed foods have exposed the weakness in traditional food control systems and accelerated the development of proactive, integrated and preventive food safety policy applicable throughout the entire food chain- from "farm or sea to table". This policy must be scientifically based, adaptive and responsive to changes in the food production chain. It should be articulated around the use of risk analysis to develop food safety objectives and standards and on the implementation of Hazard Analysis Critical Control Points (HACCP) systems. All these developments have led to very active work in the area of Codex based microbiological risk assessments including Vibrio spp. in seafoods and *Listeria monocytogenes* in ready to eat foods. These assessments are complemented with simpler "broad brush" approaches to microbiological risk assessment. The paper will discuss such methods, their practical implications and limitations. The FAO and the WHO are the principal UN agencies mandated to address issues of food safety and quality. Through Codex Committees and other expert groups FAO provides direct assistance to members, particularly developing countries, in training, advice and national capacity building. To respond to increasing demand for pertinent information on which to build risk assessments, FAO has launched the Aquatic Food Product Initiative in collaboration with the Canadian Food Inspection Agency. By fostering cooperation between various international institutions and individual scientists, the program will generate a peer-reviewed "live" safety and quality knowledge base. The Internet based tool termed FishPort (www.fishport.org) is based on the well-proven EcoPort technology. In this way multi-disciplinary knowledge in support of a food chain approach to food safety and quality is being assembled and distributed. The authors believe that this is a realistic way to assemble relevant information on the vast number of aquatic species, products and potential problem areas. The paper will discuss the major activities of the FAO Fisheries Department to assist member countries and promote international cooperation for the use of scientifically based fish safety and quality standards.

1- Introduction

Fishery products are the most traded food in the world. Thirty seven percent (live weight equivalent) of the total yearly production, estimated at around 130 million tonnes, enter international trade. Impressively, over half of this trade in value originates in developing countries, where it has become a very important source of foreign exchange, in addition to the millions of employment opportunities the fish industry provides (FAO, 2002).

Trade liberalization has been at the heart of the development agenda for several decades. Chapter 2 of Agenda 21 (Rio Di Janeiro 1992) states that the international economy should "promote sustainable development through trade liberalization". The World Trade Organization WTO classifies fish as an industrial product which carries lower import duties, as compared to agricultural products. This duty averages presently around 4.5%, whereas trade in agricultural products is still fairly limited through quotas and import duties; for example import duties for meat are commonly around 70-80%. Furthermore, the Doha round of negotiations decided that "tariff escalation" for fish and fishery products would be reduced. This means that import duties for value-added products will be lowered thus creating new opportunities, not the least for developing countries. However, these opportunities are hampered when safety and quality measures are used as disguised technical barriers to trade.

The increasing demand for fish and fishery products and developments in international fish trade has raised major concerns about overexploitation of aquatic resources. This, coupled to technological developments in food production, handling, processing and distribution and the increasing awareness and demand of consumers for safe and high quality food have put food safety and quality assurance high in the headlines. International harmonization of fish safety and quality measures, is nowadays of utmost importance to ensure the safety and quality of the traded products while avoiding unfair trade practices and disguised technical trade barriers.

2- Recent developments in fish safety and quality assurance

2-1. Statistics on fish-borne diseases and quality defects

Food safety is of major concern for food producers, traders and consumers alike. It is acknowledged that, in the few countries that report on food borne diseases, there is severe underreporting.

Recent data from the United States of America show that there have been sustained decreases in the reported incidence of four key food borne pathogens from 1996-2001 with the decreases ranging from 15% - 49%. (CDC, 2002). These decreases are reported to coincide with the implementation of new HACCP- based food safety assurance measures.

Statistics on food borne diseases (which almost only are available from developed countries) have shown that seafood is implicated in 10% - 25% of outbreaks (Nilsson & Gram 2001). Similarly recent U.S.A statistics show that some 25% of these were caused by consumption of shellfish (Olsen et al 2000). Interestingly, the same study showed that for fish, 86% of the outbreaks were caused by biotoxins (mostly ciguatera) and histamine, whereas viruses and bacteria caused 34% of the outbreaks associated with shellfish.

Likewise, consignments of fish and fishery products can be detained or rejected in international trade because of safety or quality defects. Imports to the United States (2001-2002) show that about 1/10 of the refused food products are seafoods and that the most common reason for import refusal is "filth" (some 50%), a condition which describes that the product appears to consist in whole or in part of a filthy, putrid or decomposed substance. Although details are not given for the individual products, it is assumed that microbial spoilage is the major reason for the refusal. Second in terms of rejection reason is the detection of *Salmonella* (25%). Both cooked, ready-to-eat products and raw, frozen products are rejected if *Salmonella* is detected. Although *Salmonella* has its niche in the gastrointestinal tract of birds and mammals, it is a common bacterium in ponds in tropical areas and its detection may not indicate hygienic failure. Whether or not the detection in raw foods constitute a health hazard is debatable (Huss et al. 2003).

In the European Union, the European Commission operates a Rapid Alert system for foodstuffs which is used to inform Member States about problems or risks concerning foods which do not meet food safety and quality requirements. A study (Huss et al 2003), encompassing the period from January 1999 to June 2002 indicated that the number of fish alerts has increased stadily during the period January 1999 – December 2001 and basically exploded in 2002. Chemical and drug residues (46.4%), followed by microbial contaminants (39.7%) were the main causes for alert during the period 1999-2002. The majority of alerts because of chemical and veterinary drugs residues (74.4%) occurred recently in 2002, with chloramphenicol and nitrofurans representing respectively 54% and 24.5% of the alerts caused by chemical hazards and 39.6% and 18% of the total.

Histamine and parasites caused the lowest rates of alerts, respectively 1.3% and 4%; For microbial contaminants, there was a decrease (from 59.3 % in 1999 to 41% in 2001) of alerts due to the presence of indicator organisms and an increase (from 40.1 % in 1999 to 59.2 % in 2001) of alerts because of the presence of indigenous organisms, especially *Vibrios*. The former indicates improvement in the sanitary and hygienic conditions in handling and processing fish in their countries, probably as a result of the gradual implementation of GHP/GMP and HACCP. The latter reflects more recent decisions of the EU to analyze for indigenous microorganisms, especially *Vibrio* species while awaiting the results of risk assessments of Vibrios in seafood. This assessment was finalized in 2001 and concluded that the practice of judging seafood exclusively based on total *Vibrio* counts as indicative for the presence of pathogenic Vibrios is not appropriate and should be discontinued (EC, 2001).

2-2. Developments in fish safety and quality

There has been a growing awareness of the importance of an integrated, multidisciplinary approach to food safety and quality, considering the entire food chain. FAO defines the food chain approach as recognition that the responsibility for the supply of food that is safe, healthy and nutritious is shared along the entire food chain - by all involved with the production, processing, trade and consumption of food. Stakeholders include farmers, fishermen, food processors, transport operators, distributors, consumers, as well as governments obliged to protect public health. The holistic approach to food safety along the food chain differs from previous models in which responsibility for food safety mainly concentrated on the food processing sector and government control services. The implementation of the food chain approach requires an enabling policy and regulatory environment at national and international levels with clearly defined rules and standards, establishment of appropriate food control systems and programmes at national and local levels, and provision of appropriate training and capacity building (FAO, 2003).

In the fish industry, there are five broadly defined needs on which a strategy in support of a food chain approach to food safety should be based:

- Fish safety and quality from a food chain perspective should incorporate the three fundamental components of **risk analysis** - *assessment, management and communication* – and, within this analysis

process, there should be an **institutional separation** of science-based risk assessment from risk management – which is the regulation and control of risk.

- **Tracing techniques** *(traceability)* from the primary producer (including animal feed and therapeutants used during production), through post-harvest treatment, processing and distribution to the consumer must be improved.
- **Harmonisation of fish quality and safety standards**, implying increased development and wider use of internationally agreed, scientifically-based standards is necessary.
- **Equivalence in food safety systems** – achieving similar levels of protection against fish-borne hazards and quality defects whatever means of control are used – must be further developed.
- Increased emphasis on **risk avoidance or prevention at source** within the whole food chain – *from farm or sea to plate* –, including development and dissemination of good aquaculture practices, good manufacturing practices and safety and quality assurance systems (i.e. Hazard Analysis and Critical Control Point (HACCP), are necessary to complement the traditional approach to fish safety and quality management based on regulation and control.

The principles of achieving harmonization of standards and equivalency in food control systems and the use of scientifically-based standards are embodied in two binding agreements of the WTO: the Agreement on the application of sanitary and phytosanitary (SPS) measures and the Agreement on technical barriers to trade (TBT).The SPS agreement confirms the right of WTO member countries to apply measures necessary to protect human, animal and plant life and health. The purpose of the SPS Agreement is to ensure that measures established by governments to protect human, animal and plant life and health, in the agricultural sector, including fisheries, are consistent with obligations prohibiting arbitrary or unjustifiable discrimination on trade between countries where the same conditions prevail and are not disguised restrictions on international trade. It requires that, with regard to food safety measures, WTO members base their national measures on international standards, guidelines and other recommendations adopted by the Codex Alimentarius Commission (CAC) where they exist. This does not prevent a member country from adopting stricter measures if there is a scientific justification for doing so or if the level of protection afforded by the Codex standard is inconsistent with the level of protection generally applied and deemed appropriate by the country concerned. The SPS Agreement states that any measures taken that conform to international Codex standards, guidelines or recommendations are deemed to be appropriate, necessary and not discriminatory. Finally, the SPS Agreement requires that SPS measures are to be based on an assessment of the risks to humans, animal and plant life using internationally accepted risk assessment techniques.

The objective of the TBT Agreement is to prevent the use of national or regional technical requirements, or standards in general, as unjustified technical barriers to trade. The agreement covers standards relating to all types of products including industrial products and quality requirements for foods (except requirements related to SPS measures). It includes numerous measures designed to protect the consumer against deception and economic fraud. The TBT Agreement basically provides that all technical standards and regulations must have a legitimate purpose and that the impact or cost of implementing the standard must be proportional to the purpose of the standard. It also states that, if there are two or more ways of achieving the same objective, the least trade restrictive alternative should be followed. The agreement also places emphasis on international standards, WTO members being obliged to use international standards or parts of them except where the international standard would be ineffective or inappropriate in the national situation. The aspects of food standards that TBT requirements cover specifically are quality provisions, nutritional requirements, labelling, packaging and product content regulations, and methods of analysis. Unlike the SPS Agreement, the TBT Agreement does not specifically name international standard setting bodies, whose standards are to be used as benchmarks for judging compliance with the provisions of the Agreement.

Risk analysis is widely recognized today as the fundamental methodology underlying the development of food safety standard that provides adequate health protection and facilitates trade in food (FAO, 2001). There is a fundamental difference between a hazard and a risk. A hazard is a biological, chemical or physical agent in, or condition of food, with the potential to cause an adverse health effect. In contrast, risk is an estimate of the probability and severity in exposed populations of the adverse health effects resulting from hazard(s) in food. Risk analysis is a process consisting of three components: risk assessment, risk management and risk communication. Risk assessment is the scientific evaluation of known or potential adverse health effects resulting from human exposure to food-borne hazards**.** Risk management is the process of weighing policy alternatives to accept, minimize or reduce assessed risks and to select and implement appropriate options. Risk communication is an interactive process of exchange of information and opinion on risk among risk assessors, risk managers, and other interested parties.

The responsibility for the supply of fish that is safe, healthy and nutritious should be shared along the entire chain from primary production to consumption. Development and implementation of Good Aquaculture Practices (GAP), Good Hygienic Practices (GHP) and Hazard Analysis Critical Control Point (HACCP) are required in the

food chain step(s). Government institutions should develop an enabling policy and a regulatory environment, organize the control services, train personnel, upgrade the control facilities and laboratories and develop national surveillance programs for relevant hazards. The support institutions (academia, trade associations, private sector, etc.) should also train personnel involved in the food chain, conduct research on quality, safety and risk assessments, and provide technical support to stakeholders. Finally, consumers and consumer advocacy groups have a counterbalancing role to ensure that safety and quality are not undermined by political considerations solely when drafting legislation or implementing safety and quality policies. They also have a major role in educating and informing the consumer about the major safety and quality issues.

The general principles of GHP/HACCP have been adopted by the Codex Alimentarius Commission (CAC) in 1997 and 1999 (FAO, 2001). They include requirements for the design and facilities, control of operations (including temperature, raw materials, water supply, documentation and recall procedures), maintenance and sanitation, personal hygiene and training of personnel. Similarly, the Codex Committee on Fish and Fishery products is working on a draft code of practice for fish and fishery products, including aquaculture products, which integrates these general principles and adapts them to aquaculture [(2)]. This Code is not intended to cover extensive fish farming systems or integrated livestock and fish culture systems that dominate production in many developing countries.

Control and prevention of chemical pollutants and biotoxins require the implementation of appropriate monitoring and surveillance programs. This is particularly important for mollusc culture, filter feeders that can concentrate pollutants, biological agents, and biotoxins. The Codex Code of Practice describes the requirements for surveys and monitoring of the harvesting and growing areas to determine sources of domestic and industrial pollution, classification of the areas into suitable for harvesting, relaying or non-suitable for growing or harvesting, and the frequency and methods of monitoring.

3- Role of FAO and other stakeholders

FAO's normative work in food safety and quality is focused on food standards linked to the Codex Alimentarius and developed in close collaboration with WHO, and related capacity-building. Codex Alimentarius includes standards for all principle foods (whether processed, semi-processed or raw) for distribution to the consumer, with provisions related to food hygiene, food additives, pesticide residues, contaminants, labelling, presentation, methods of analysis and sampling. The Codex Secretariat is housed in the FAO Food and Nutrition Division (ESN), which has primary responsibility for normative work in food safety. FAO in collaboration with WHO provides expert scientific advice for standards setting through Codex expert committees and/or meetings.

In addition to its contribution to the normative work of FAO, through the participation to the relevant Codex committees and the support to the work on risk assessment for fish and fishery products, the FAO Department of Fisheries (FI), provides direct assistance to member countries for the application of good farm management practices and the implementation of the FAO/WHO fish standards, guidelines and Codes of practices. FI's assistance to member countries centres around training of personnel from government authorities and the fish industry, provision of technical advice on legislation and organization of fish inspection and building national capacity in fish quality and safety research.

3-1 Training and technical assistance

Mainly through extra-budgetary funding received from Denmark, but also through the TCP funding mechanism of FAO, FI was able to carry out over 60 workshops and train more than 1500 professionals from industry and the Government in HACCP-based fish quality and safety systems since 1995. This training, coupled with the provision of technical advice have enabled many developing countries to meet safety and quality requirements of international markets. For example, the 2002 EU list I of third countries from which import of fish for human consumption is authorized totalled 73 of which 52 were from the developing world, including 18 from Africa (EU, 2003). However, the need for further training is ever increasing, especially in the areas of HACCP implementation and assessment and risk analysis. In this respect, FI is seeking extra-budgetary funds to meet the increasing demand in these fields.

FI was also involved in the FAO Umbrella training programme on Multilateral trade negotiations in agriculture, fisheries and forestry, especially in regions where fisheries are important. An initial series of fourteen sub-regional workshops were completed by July 2001: four in Africa, three in Asia, two in the Near East, two in Europe and three in Latin America and the Caribbean. These workshops deal in a comprehensive way with the SPS/TBT agreements. This programme was supplemented with a series of 5 training workshops on the implications of the WTO agreements on fish trade, organized by FI through its Info Services in Asia, Latin America and Africa. An umbrella II programme has been prepared recently and submitted to donors. It has a stand alone fisheries component that aims at building capacity, including through training, on SPS/TBT and on the application of risk assessment in the fish industry.

It is important to point out that FI's field activities in training and technical assistance enable its staff to review the practical implications resulting from the implementation of food safety policies for fish and fishery products and contribute the results to the adaptation/review of the FAO/WHO work, including emerging fish safety issues and new developments in fish production, processing and distribution.

3-2 Contribution to the CAC work

FI participates to the work of the Codex committee on fish and fishery products (CCFFP) and to the work of the Codex Committee on food hygiene (CCFH), especially its FAO/WHO work on risk assessment of microbiological hazards in foods.

The CCFFP, which is hosted by Norway meets every two years and deals with standards and guidelines relating to fish and fishery products. Currently the most important issue under discussion at the CCFFP is the proposed Recommended Code of Practice for Fish and Fishery Products. The proposed Code introduces all over the board the concept of HACCP, for safety of fishery products , and also proposes a basic quality control along the same concept. The Committee assigned to FAO and WHO the drafting of the chapter dealing with aquaculture products, extended (in the case of FAO) to the handling of live fish. Other technical documents (Use of chlorine in water in contact with fish, fish identification and authentification using molecular biology techniques) on specific areas to be presented for the consideration of the CCFFP are also prepared by FI.

In the area of risk analysis, risk assessment of microbiological hazards in foods has been identified as a priority area of work for the CAC. At its 32nd session in 1999, the Codex Committee on Food Hygiene (CCFH) identified a list of 21 pathogen-commodity combinations that require expert risk assessment advice. In response, FAO and WHO, jointly launched a programme of work with the objective of providing expert advice on risk assessment of microbiological hazards in foods to their Member countries and to the CAC. The two risk assessments of interest to fish and fishery products are *Listeria monocytogenes* in ready to eat foods, and *Vibrio* spp. in seafoods, which now are in their final phases. It is clear that such quantitative risk assessments are very demanding in time and money. Therefore, a "broad brush approach" to assess and rank chemical and microbiological hazards in foods would be a good way to start systematising such approaches. FI has prepared a document to fill this need (Sumner et al.2003). The document works through examples of how risk analysis can be used for different reasons and provides back-up resources for situations without extensive library or on-line facilities. The document includes also a spreadsheet tool, Risk Ranger (Sumner and Ross, 2001), which helps in doing semi-quantitative risk analysis and risk profiles. The Risk Ranger" assignes a ranking figure to the products from 0 – 100. Low risk ranking is < 35, Medium Risk Ranking is 39 – 59 and High Risk Ranking is > 60. It is hoped that this document can become an important tool in training risk assessors and risk managers by systematically going through the steps of risk assessment. The approach is particularly useful for determining where to place emphasis on control measures such as in determining Critical Control Points in a HACCP plan. It is also excellent for training purposes as it makes clear what data are required (e.g. how prepared prior to eating, sizes of portions, epidemiological data, immunoprofile of the population, etc.) and thus "demystifies" the whole process. It will undoubtedly take considerable time before results of full quantitative risk assessment are expressed in food safety legislation, standards and guidelines. In the meantime the Sumner-Ross method could become a valuable tool.

3-3. Fish safety and quality knowledge base – FishPort

Food hazards vary greatly in origin and type particularly for products originating in the aquatic environment which span the whole range of invertebrate and invertebrate species including plants. To conduct a proper hazard analysis it is necessary to integrate information from several scientific disciplines to implement adequate controls or mitigation strategies. Although the internet offers a wide range of scientific information, finding adequate and succinct information can be a perplexing experience for the novice user of the internet. Today, the delivery of information is less of a challenge than updating and linking of accurate and pertinent information. One way to achieve this is to have on-line active participation of the scientific and technical community. The concept of using a "Food Continuum" (Blaha & Stohr, 1999) implies the integration of causes and sources of hazards from the production to the consumption. (McEachern & Mountjoy, 1998). An integrated approach in terms of risk management for the entire food chain is therefore the logical next step in the product of safe and acceptable food products.

To work towards such an integrated internet based system for aquatic products, FAO has launched the Aquatic Food Product Initiative in partnership with other international organizations. The initiative takes a long term view and is seeking participation of scientists worldwide to create an integrated knowledge base on safety and quality of aquatic food products.

In the long term this knowledge base is expected to contribute to a more level playing field between developed and developing countries in activities such as hazard analysis, risk assessment, quality evaluation and product development. It will create a knowledge base of synoptic scientific and technological information for practical application and training. Contributions are submitted through password protected access and presented

under the name of his or her institution, both as a means to ensure proper recognition for the work and to ensure appropriate level of quality assurance. In addition, the author and the institution assume the maintenance of their information. Under the supervision of FAO, all contributions are peer reviewed and all texts must be fully cited.

The FishPort website (www.fishport.org) uses "EcoPort"technology which has been developed for over 10 years in close collaboration with scientists mainly involved in plant pest and crop management (www.ecoport.org). It operates under the auspices of the FAO, the Smithsonian Institute and the University of Florida. EcoPort is a species based system that combines the biological information of species within an ecological context. FishPort uses the species information in the form of entities that are designed to describe food hazards food quality attributes, processing methods and products. Today, EcoPort has some 1200 contributors from 130 institutes and universities around the world. It currently holds some 35,000 photographs and almost 600,000 references.

FII is contacting scientists and institues to contribute and act as "gatekeepers" and contributors in the area of safety and quality of fishery products with plans to reach to processing methods and composition of products at a later stage

Conclusion

The globalization and further liberalization of world fish trade, while offering many benefits and opportunities, also presents new safety and quality challenges. Despite improvements made in this area, improved scientific tools must be adopted and novel flexible approaches to safety must be sought so that regulatory actions can reflect the most current scientific evidence and that responsibility for safety is effectively shared along a food chain.

Fish safety and quality assurance in the new millennium will require enhanced levels of international co-operation in promoting harmonisation, equivalency schemes and standards setting mechanisms based on science. The SPS/TBT agreements of the WTO and the benchmarking role of the Codex provide an international platform in this respect.

Important reforms to tackle these issues have been initiated in the USA (NAS, 2003), the EU (2000) and many other countries. Unfortunately, developing countries are at a disadvantage because of insufficient/inadequate national capacities and resources. International organizations such as FAO must revamp their programmes and seek the necessary resources to assist in this endeavour.

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R 1. THE FISH TECHNOLOGY KNOWLEDGE BASE - FACILITIES AND OPPORTUNITIES FOR NETWORKING FOR FISH TECHNOLOGISTS

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Summary

A Fish Technology Knowledge Base in the oneFish Community Directory (www.onefish.org), an open-access Internet portal for all fisheries research, was set up in response to fish technologists' views worldwide. oneFish, supported by FAO, is an established platform, suitable for hosting a global fish technologists' network. Some 38,000 bibliographic records and other documents covering most aspects of fish technology, and linked to the Utilization and Technology Worldview in oneFish, form the core of the Knowledge Base. New records are being added as part of the EC project QLRI-CT2000-00216 and the FAO-SIFAR work programme. It is now possible for organisations and individuals to rapidly export to the Knowledge Base records (single, multiple or database) in a suitable format. This new facility makes it much easier to up-date the Knowledge Base, encourage expansion of the Network of contributors, and enhance the information available to users.

Introduction and Background

The importance of global flow of information and networking in fisheries and aquatic R&D is increasing in importance due to globalisation of the fish resource and environmental and safety issues. The need for information is particularly acute in developing countries. Fish technology underpins the international trade in fisheries products worth more than US $110 billion annually. There is also a need to avoid unnecessary and expensive duplication of R&D and services as a result of inadequate information being available, and to ensure that the full benefit is derived from information already in the public domain or otherwise available. A survey (Whittle and Nesvadba, 1999) of the views of fish technologists from 30 countries was undertaken by the Fisheries Industry Division, Food and Agriculture Organisation (FAO) of the UN in 1999. The survey confirmed a strong interest in the development of a specialist fish technology knowledge base, especially one that would allow greater access to the so-called 'grey' literature. This was reported briefly by FAO at the 29th WEFTA Meeting in Greece in 1999, together with news of the imminent development of a new Web-based knowledge management system for the fisheries and aquatic research community, known as oneFish.

What is oneFish?

In 2000, the Fisheries Department of FAO in partnership with WAICENT (World Agriculture Information Centre) and SIFAR (Support Unit for International Fisheries and Aquatic Research) began to develop oneFish, an open community directory, designed as a single internet portal for all fisheries research. It allows registered users across the whole fisheries and aquatic research community to contribute information in any electronic media that can be uploaded and linked to specific subject areas, or Topics, in oneFish (including Utilization and Technology). Books, documents, references, websites, maps, contacts, projects, news and other relevant electronic data can be stored as Knowledge Objects (KOs). The system allows users to search and retrieve information using keyword(s), author(s), or other criteria, identify links to other sources of relevant information, be alerted to related topics of interest, and have access to a current news digest in many areas of aquatic research. For the fish technologist, oneFish can correlate and disseminate information via the Topic Tree, Free Text and other media, and linkages to a Fish Technology Knowledge Base and Network. There are facilities for special interest groups to develop discussion groups and Forums, and Virtual Offices can be created for groups, institutes and other organisations. These provide excellent facilities for Networking. Management of oneFish is devolved. Specialist subject Topic Editors volunteer to be registered in oneFish to assist in managing specific topics and control the content and quality of their particular topic areas. The system went live in March 2001 at www.onefish.org and is continually being upgraded to improve the facilities in response to user feedback. At the time of writing (April 2003), oneFish has about 52,000 KOs, 940 Members, 70 Editors and 980 Topics.

The objectives of the project

The primary aim of the project is to enhance the knowledge infrastructure for fish processing technology and related areas, facilitate more effective development of R&D and application of technology, and achieve better quality and safety of fisheries products. Since April 2001, with funding from the EC (QLRI-CT2000-00216), our Consortium of participants in FAO, SIFAR, Iceland, Poland, Portugal and the UK has been working to establish the post-harvest Fish Technology Knowledge Base in oneFish. We have also been working to develop a co-operative Network (currently standing at 10 members) from R&D institutes, industry and others, to contribute to

and expand the Knowledge Base. FAO undertook the responsibility to remain as system host, maintaining the system on behalf of the Network after completion of the EC project, and to be responsible for globalisation.

The Fish Technology Knowledge Base

The oneFish software and website was enhanced to facilitate incorporation and development of the Fish Technology Knowledge Base and the Network that will contribute new information to the knowledge base. A Virtual Office was set-up for the project in oneFish that can be accessed directly from the oneFish homepage www.onefish.org, with institutional topics for each project partner. The Fish Technology Knowledge Base or the Utilization and Technology Topics are just one click away from the oneFish homepage.

The Fish Technology Knowledge Base defines fish technology as broadly as possible to cover the wide variety of R&D and application work going on in fish technology institutes, industry and academia world-wide, in order to maximise the utility of the Knowledge Base. The scope of this definition is described in the fish technology Topic Tree and Sitemap in Utilization and Technology, that spans the Sub-topics: Resources, Shellfish growing waters, Harvesting, Composition and properties, Onboard handling, Preservation methods, Handling and processing, Food products, Food product safety, Food product quality, Non-food products, Distribution and storage, Technology transfer, Markets and trade, Economics, and Policy, law and regulations. The Sitemap expands the topics to 250 fish technology headings. Knowledge Objects can be added to a Topic with links to other topics using the versatility of the oneFish system. Some 2110 Keywords relevant to fish technology have now been listed to support development of the Knowledge Base, and a preliminary Glossary of definitions of fish technology terms, with some 630 entries, is also available. Both are accessible directly from the oneFish homepage. Selected postings from the Seafood Listserve of UC Davis provide additional sources of discussion and information.

A specialist fish technology bibliographic database, derived from the database (TORLIB), established at Torry Research Station, Aberdeen, in 1984, was introduced as the ready-made core to kick-start the Knowledge Base. Originally, this was made available on the Internet as Nelfish to demonstrate the content of the database and is now integrated into oneFish. This large database includes some 38,000 records of references and abstracts on many aspects of fish technology and related topics, including key publications dating back to 1947. These records have the major advantage that they were filtered and selected for inclusion in the database by fish technology specialists, making the database a unique and very significant, valuable collection of information. Nearly 30% of the 29,000 journal records were 'cherry-picked' from over 2,400 journals outside the mainstream food science and technology literature, emphasising the advantage of input to the database by active researchers, and it is an advantage that we would like to see maintained. The database provides easy access to the important peer-reviewed and 'grey' scientific and technical literature on fish technology topics, gathered together over 5 decades, to assist researchers both in their research work and to identify potential solutions to processing problems raised by industry. Arrangements have been made with Central Science Laboratory (York, UK) to make available unpublished Torry reports that are recorded in the database.

The power of TORLIB was tested (Whittle and Nesvadba, 1999) by comparing it with the professional / commercial databases ASFA and FSTA over 16 wide-ranging search topics in fish technology covering the years 1990 to 1995. TORLIB produced the best search results of the 3 databases. It found the most relevant records overall and the most journal records overall. It gave the best results (in terms of relevant records found) for 7 search topics and the worst results for only 3 topics; ASFA was best for 6 topics and worst for 6 topics; FSTA was best for only 3 topics and worst for 7 topics.

The historical value, size, significance and power of TORLIB as a pre-existing, specialist fish technology database made it an obvious and logical candidate to provide a ready-made core of information to kick-start a new Fish Technology Knowledge Base, accessible to all, within the powerful and very versatile facilities of the oneFish knowledge management system.

The Fish Technology Knowledge Base is established, operational and fully accessible. It now has a substantial core of data. Fish technology records are continually being added to oneFish and linked to relevant topics in the Fish Technology Topic Tree by the nucleus of a Network (currently 10) provided by the participants in this project as well as new contributors. FAO have supported this approach. To maximise the value of the database, new, up-to-date and reliable information needs to be added on a continuous basis, and the fish technology content needs to be broadened and enhanced. 640 WEFTA papers have been extracted from the TORLIB database and converted for import into a specially created WEFTA topic in oneFish, and there is a link to the WEFTA website.

Enhancing and sustaining a Network of users and contributors of data and information

We recognise that the success of the Knowledge Base depends on three main factors:

- It must provide users with what they need, and evolve as needs change.
- The content of the database needs to be continually up-dated.
- It needs to be supported and sustained by development of a much larger co-operative network of both contributors and users from research and industry.

A new development in oneFish will make addition to the database and enhancement of the contributory network much easier, less time consuming and automatic. It will be possible to import to oneFish a collection of references or Knowledge Objects, i.e. a mini-database (called a KO Pool) held in another system. A query can be

sent to the other system, and the metadata of all the relevant KOs are retrieved and stored in a KO Pool in a format that matches that of oneFish KOs. When a user searches oneFish, not only all of the KOs in oneFish are searched, but also all of the KOs referenced in any linked KO Pools. In this way, Institutes or individuals will be able to automatically contribute a list of recent publications and abstracts or reports, or other information to the Knowledge Base. This latest feature will provide oneFish users with access to a much wider range of resources relevant to fisheries and aquatic research, via the oneFish interface.

The Future development of the Knowledge Base Network

Established and running effectively as a result of this project, our intention is to devolve inputs to the Knowledge Base to user centres and individuals in line with the philosophy of oneFish. Subject specialist, volunteer, Topic Editors will help manage specific topics and be responsible for the quality and content of their own topic areas. Importantly, FAO guarantees to continue its responsibility as the system host or provider and maintain the Knowledge Base on behalf of the established Network after conclusion of this project. The long-term objective for FAO, beyond the scope of this project, is the establishment of a global network on fish technology with reliable inputs to the Knowledge Base provided by contributors on a worldwide basis.

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Innovative handling and processing of fish and shellfish.

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First Joint Trans-Atlantic Fisheries Technology Conference - TAFT 2003
33th WEFTA and 48th AFTC meetings,
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K 1. MINIMAL PROCESSING TECHNOLOGIES IN THE FOOD INDUSTRY

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Abstract

Minimal processing of foods covers a wide range of technologies and methods, for preserving food during their transport from the agricultural production to the consumer. Minimal processing methods have in common that the processing changes the inherent fresh-like quality attributes of the foods as little as possible (minimally), while the processing gives the food product sufficient shelf-life for the transport from processing to consumers. Minimal processing technologies can be applied in various stages of the food production chain, in storage of raw materials, processing, packaging and distribution. In this presentation the various methods will be presented and recent developments reviewed; including post-harvest technologies, non-thermal and mild thermal processing methods, modified atmosphere and active packaging. The possibilities for their application to seafood products will also be discussed.

Introduction

Minimal processing of foods covers a wide range of technologies and methods, for preserving food during their transport from the agricultural production to the consumer. Minimal processing methods have in common that the processing minimally changes the inherent fresh-like quality attributes of the food, while also giving the food product sufficient shelf-life for the transport from processing to consumers. Sometimes the term "Invisible (to the consumers) processing" is used for some of these methods. Another definition which situates the minimal processing methods within the context of conventional technologies describes them as techniques that preserve foods but retain the nutritional quality and the sensory characteristics by reducing the reliance on heat as the main preservation action. The interest in minimal processing technologies is very much a response to the demands expressed by the consumers for products that are fresh and more like the natural products as the highly processed foods are seen as lower in nutritional and sensory quality. Another important consumer trend to consider is the concern that the consumers have towards food additives. The recent food scares on high levels of acrylamide in foods and previous scandals about contaminants in feeds have most probably increased the uncertainty and the need for guarantees among the consumers. There is a feeling that few foods are safe, which results in reluctance to trust the traditional food supply and especially "new" foods. A very important food product market trend, which also must be brought into the development of modern food processing methods, is the demand for convenience with products which need less time to prepare and are ready to serve or ready to cook or reheat.

The most active market segment is the chilled, ready-to-eat or ready-to-heat foods; typically in single serving packages, designed to meet the needs of convenience and simplicity. But many of these products have short shelf lives, typically a few days only limiting the geographical area where they can me marketed. Technologies that allow for a two- or threefold prolongation of the shelf life are much sought after. It is not only the extended time of distribution and storage that is attractive. Also, added product safety is much in demand by producers and distributors. The fresh-like products are highly perishable, and actions that add to the safety factors are important as seen both by the food processor and the retailer.

On the other side of the shelf life spectrum, the traditional long-life ambient storage products are also affected by the consumer trends. Long shelf life is no longer an important selling point, with the market trends towards more fresh-like products. Therefore, preservation technologies that prolong shelf life but do not act detrimentally on the product quality attributes are in favour on the market. The push towards shorter shelf lives for ambient shelf-life products is also supported by the demand for more rapid turnover of the stock.

In conclusion, products with a very short shelf-life will need preservation methods that will prolong the shelf life, and the long shelf- life products need methods that give shorter shelf life but with improved quality. These methods are to be found in the group of technologies and methods that are called minimal processing.

The objective of this review is to present a range of minimal processing technologies following the food distribution chain. More detailed accounts of these methods can be found in the literature, (Singh and Oliveira, 1994, Wiley, 1994 , Ohlsson and Bengtsson, 2002). For the minimal processing technologies presented in the following a key reference for further studies is given. In the presentation the recent developments in the area will be reviewed with particular emphasis on existing or possible application in the seafood sector.

Minimal Processing Technologies

In minimally processed foods, storage, processing, packaging and distribution is accomplished in highly integrated systems. Each step must be considered in conjunction with the other steps. Various minimal processing methods can be applied in the different steps in the food distribution chain from storage of raw materials to

processing and packaging of the consumer product. A list of the methods that will be shortly reviewed here is given in Table 1.

Table 1 Applications of minimal processing technologies

Process	Applications	Mechanisms
Controlled atmosphere storage	Bulk-stored fresh fruit and vegetables	Altered respiration rates Antimicrobial effect
Post-harvest treatments	Fruits and vegetables	Antimicrobial and antioxidative effects
Clean-room technologies	Fresh meat and fish Prepared foods	Reduced levels of pathogen micro-organisms
Protective microbes	Dairy products	Release by bacteriocins that reduce micro-organisms
Non-thermal processing methods		
- High pressure treatment	Fruit and vegetable products, ham, oysters	Cell membranes ruptured
- Irradiation	Fresh fruit, meat, poultry and spices	Ability of microorganisms to reproduce eliminated
- High electric field pulses	Fruit products	Microbial cell membrane rupture
- Surface decontamination UVC Pulsed light NIR	Food and production area surfaces	 DNA damage DNA damage + heat? Surface heat
New thermal processing methods - Ohmic heating - High-frequency heating - Microwave heating	Many products, prepared foods	Direct volume heating - Sufficient microbiological effect while minimizing thermal quality changes
- Sous-vide technology	Prepared foods	Mild and controlled heating and cooling
New packaging technologies		
- Modified-atmosphere packaging and active packaging	Fresh meat and fish, prepared and baked foods fresh fruit, vegetables	Antimicrobial effect altered respiration rates
- Edible films	Dry, frozen and semi-moist foods	Protection against oxygen and moisture loss

Modified atmosphere storage

By modifying the composition and sometimes the overall pressure of the atmosphere in the storage environment, quality- and safety-degrading biological reactions can be slowed down or inhibited. The major application is for respiring fruits and vegetables, where increased carbon dioxide concentration (up to 10%) and reduced oxygen concentration (3-5%) will retard respiration and prolong the shelf life. This application together with the post harvest methods presented below is where Minimal processing really started, (Wiley, 1994). Relative humidity is also an important factor as is control of ethylene emission from the respiring fruits and vegetables.

Post-harvest treatments

In order to add convenience to vegetables and other agricultural products; centralised cleaning, peeling and cutting is common. The resulting products are often less stable after the treatment, due to enzymatic activity of cut cell walls and bacteriological contamination from the handling. The major driving force is the added convenience to the consumer. Various post-harvest treatment methods are employed to add biological stability and extend their shelf life. Chlorinated cleaning water is used. Soaking in solutions of reducing agents such as ascorbic acid or sulphite; or preservatives such as sorbate or benzoate is used. Also divalent ions, Ca^{++}, are used to strengthen the

texture. In all these treatments low temperature and good processing hygiene are essential to achieve the desired shelf life, as treatments such as cutting instead reduce the shelf life, (Laurila and Ahvenainen, 2002). Often especially design packaging is used for theses products, allowing some of the carbon dioxide produced during respiration to permeate out of the package while a sufficient fraction remains in the package to give a retardation of the enzymatic and microbiological activity during storage.

Hurdle technology

Reducing the levels of salt, sugar or acid in foods in order to improve consumers' acceptance often means that the food is more perishable. To attain sufficient shelf life, combination of preservation methods is often applied. The principle is that one of the preservation actions, the hurdle, do not give enough protection to microbiological growth, only by adding the effects of a number of hurdles is the desired shelf life extension achieved. This methodology of combining preservation treatments is nothing new in principle. Many of out traditional preservation methods are based on such combinations. Modern hurdle technology studies the interactions of the various preservation steps on microbiological and quality development during storage. (Leister and Gorris, 1995). By fine-tuning the preservation system with a number of hurdles or methods, more knowledge is built into the product; an important step in product development.

Clean room technologies

The objective in clean room technology is to eliminate microbiological contamination on the products mostly coming from humans or from the environment. In the handling of the food contact between humans and the foods are eliminated as much as possible. Production personnel use extensively protective clothing; all to reduce air-, and human-, borne micro-organisms. Equipment and the processing environment are properly cleaned and disinfected prior to production. The equipment and the surface materials used inn the processing hall should be selected with hygiene and cleanability considerations. Air curtain and a positive air pressure are maintained in the processing hall which must be built with ventilation systems using filtered air. Clean room technologies are primarily applied today for fresh prepared foods, some meat and dairy products. Clean room technology is often an expensive method, which often means that the application is limited to high value added products and to a limited part of the production line. Hygienic design and hygienic processing considerations should always be incorporated in the planning of food production also of minimally processed foods, (Lelieveld and Mostert, 2003).

Protective microbiological treatment

It is well known that many micro-organisms produce anti-microbial agents. Some lactic acid producing bacteria produce bacteriocins, that are efficient in stopping the growth of, in particular, Gram-positive spoilage bacteria. By adding selected lactic acid- producing strains to the surface of foods, controlled growth can create an antimicrobial condition at the food surface. Only a few commercial applications of natural antimicrobials are presently known, in particular the application of lysozyme and Nisin to dairy products, (Meyer et al, 2002).

Non-thermal processing

Ultra high pressure

By applying pressures in the range of some thousand atmospheres micro-organisms and enzymes can be inactivated without the degradation of flavours and nutrients associated with traditional processes. Under the ultra high pressure the material is compressed and the hydrogen bonds of the water molecules are affected. Microscope studies reveal that cell membranes are broken during the ultra high pressure treatment. Vegetative microbial cells are inactivated at pressures between 300 and 600 MPa. Inactivation of bacterial spores requires higher pressures and combination with elevated temperatures. The combination of ultra high pressures above 700 MPa and 90 degree C is claimed to give commercial sterility to low acid foods, with limited changes of sensory and nutritional quality, (Hoogland at al, 2001). Many enzymes are inactivated at pressures in the range of 300 to 700 MPa, but some are more stable.

High-pressure treatment also changes the texture of food. Proteins structures are damaged at pressures above 100 MPa, as shown for example in studies on fish muscle proteins. Irreversible changes and coagulation occurs at pressures above 400 MPa. Other biopolymers are also affected at higher pressures, e g polysaccharides form gels. The textural changes induced by high pressure treatment create different, often more dense structures than thermal treatment. If is sometimes said the pressure treatment brings another dimension to structure formation in addition to time and temperature.

The method is commercialised in Japan and Europe for treatment to prolong the shelf life of low pH fruit products. In Spain, ham products are treated with a resulting prolonged shelf life. In the USA, avocado paste is treated at very high pressures. It has also been found that pressure treatment at 300MPa affects the main muscle of oysters, making the opening much easier. High pressure treated oysters are now introduced on the US market, (Barbosa et al, 1998).

Irradiation

Very extensive research into the method in the 50´s and 60´s; particularly the wholesomeness of the irradiated products demonstrated that the method is an efficient and safe preservation method. According to WHO, FDA and USDA the great virtue of irradiation is that the process effectively inactivates disease-causing bacteria without negative effect on food quality. In spite of this, the commercial use has been very limited, due to consumer scepticism coupled to legislative limitation. There is a gradually increasing use of the method in the USA, with applications for fresh fruits, fresh meat and poultry using radiation doses in the range up to 5 kGy. For disinfection of spices, irradiation is allowed and used in a large number of countries. The method is expensive, with typical processing costs of 0.1-0.2 €/kg product. Of course this limits the application to high value products.

High electric field pulses (PEF)

When biological cells are subjected to electrical pulses of high field strength, cell membranes are broken due to an uneven distribution of electrical changes on both sides of the cell membranes. For inactivation of micro-organisms electrical field strength of 25 35 kV/cm is needed, whereas for perforation of vegetative and muscle cell walls electrical field strength in the range of a few kV/cm is sufficient. The efficiency of microbiological inactivation is depending on a number of other factors, e g the size of the cell, the pulse shape and duration, the conductivity of the surrounding medium. Enzymes and proteins are not affected by the treatment. However secondary effects of the PEF treatment are found from the leakage of cell material, (Barbosa et al 1999).

As the temperature increases somewhat due to conductivity heating during PEF treatment, the selection of pulse duration and number of pulses must be selected to optimise the desired effect. A small number of industrial scale PEF units are now tested, primarily for applications of "cold pasteurisation" of fruit juices and other drinks. The application of PEF treatment of solid food has mainly been focused on improving the extraction yields after that the cell walls have been perforated. In the seafood sector, PEF treatment has been investigated for improving the extraction yields of seaweeds.

Surface decontamination

Most of the bacteriological contamination is found on the surface of the foods. It is therefore of interest to find methods which decontaminate the surface area of the foods by inactivating micro-organisms without affecting the sensory or nutritional quality of the foods. UV-C light at 1 kJ/m^2 inactivates bacteria, yeast and viruses by as much as 4 log reductions. UV-C light is used today for disinfecting production areas and equipment. The application on foods is now studied, starting with mould reduction on bread.

A similar application area is found for pulsed white light. The high current discharge through gas filled flashlights results in millisecond flashes of broad spectrum white light, about 20 000 times more intense than sunlight. The treatment time is very short making it suitable for application on continuous production lines. Again the applications are mainly for production areas and packaging material, but studies are under way for the application on food surfaces. The bacteriological reduction at permitted energy flux levels for foods, such as fish and meat, is typically only 2-3 log cycles.

Although it is mostly a thermal method, it should be mentioned here that surface decontamination is also performed intensive NIR in short flashes of 2- 5 second. The surface temperature rises rapidly and the surface decontamination is killed off, but the bulk of the food is heated to a small extent. The major application is for surface decontamination of cereal products, (Ohlsson and Bengtsson, 2002).

Thermal processing

Volume heating methods

The classical approach to overcome or at least minimise the undesirable quality changes in thermal processing is the HTST (High Temperature Short Time) concept. It is based on the fact that the inactivation of micro-organisms primarily depends on the temperature of the heat treatment, whereas many undesirable quality changes depend primarily on the time duration of the heat treatment. To accomplish HTST-processing, there is much interest in mild heat treatment methods, which avoid excessive temperatures resulting in thermally induced quality losses. Often a reduction of only a few degrees can have a dramatic influence on e g liquid losses of meat or fish. Yet the heat treatment needs to give microbiological safety to the product. With the help of modern process optimisation and control methods, mild heat treatments that combine these objectives are developed. Among these, most interest is found for direct volume heating methods such as ohmic, high frequency and microwave. These heating methods can be used to quickly raise the temperatures of the whole volume of the food and thus shorten the processing time.

In these electric heating methods, it is important to have an understanding of the interaction between the electromagnetic field at the frequency in question and the food material being subjected to the energy. Knowing the electric and dielectric properties of food and other materials in the heating equipment is important in order to better understand and control the application of electric energy for the heating of foods.

In ohmic or electric resistance heating, the food itself acts as a conductor of electricity taken from the mains supply. The food may also be immersed in a conducting liquid, normally a weak salt solution of similar conductivity to the food. Normally, voltages up to 5000 V are applied. The ohmic heating system shows excellent retention of particle integrity for particles up to 2 cm diameter, due to the absence of mechanical agitation, typical for traditional heat exchanger- based systems. A wide range of particle containing foods can be processed in ohmic heating systems. However the number of commercial installation is limited.

High frequency (HF) heating is done at frequencies of 13,56 and 27,12 MHz. Foods are heated by transmitting electromagnetic energy through the food placed between an electrode and the ground, also over air gaps and through non-conducting packaging materials. To achieve sufficiently rapid heating in foods, high electric field strengths are needed.

Both high frequency and microwave heating is accomplished by a combination of dipole heating, when the water dipole tries to align itself with the alternating electric field, and electric resistance heating from the movement of the dissolved ions of the foods.

The major application for high frequency heating in the food industry is for finish drying of cereals products. A growing, application of HF heating in the food processing industry is its use for the bulk defrosting of fish. The volumetric nature of HF heating allows the thawing process to be accelerated, whilst still maintaining control of the temperature distribution within the food product. Typically, HF defrosting times of 1-2 hours are possible.

Microwaves used in the food industry for heating are the ISM (Industrial, Scientific and Medical) frequencies 2 450 MHz or 915 (896) MHz, corresponding to 12 or 34 cm in wavelength. The penetration ability of microwaves in foods is limited. For normal "wet" foods the penetration depth is approximately 1 to 2 cm at 2450 MHz. The limited penetration depth of microwaves implies that the distribution of energy and heat within the food can vary. The control of the heating uniformity of microwave heating is difficult, which also has been a clear limitation for industrial application of microwave heating, (Ohlsson and Bengtsson, 2001). Today HF and microwave equipment is designed by computerised methods to control the heating uniformity, e g by controlling the over heating of edges and corners of the foods in microwave heating. The direct electric heating methods are more expensive than traditional heating methods. Thus their use is limited to processes with benefits in terms of better production yield or product quality. The major application areas are for partial thawing (tempering) of frozen blocks of meat and fish, for pasteurisation of packaged convenience foods and bread and for microwave assisted drying of cereals and other particular foods, (Ohlsson and Bengtsson, 2002).

Sous vide

The actual cooking of raw foods under vacuum, in the package, so called “sous-vide” cooking, has been developed in France and has gained fairly wide acceptance as a means of producing high quality cooked foods of limited refrigerated shelf life. As the name implies, the raw material is packed under vacuum, in multilayer plastic packaging, and cooked in water, by air/steam mixtures or by microwaves at temperatures below 100°C, removing oxygen. It is cooled rapidly to +3°C and then stored at 0-+3°C. Loss of nutrients is minimised and an excellent quality obtained. A wide range of vegetables, meat and fish products are being processed by this technique, primarily for the institutional market but also increasingly also the consumer markets.

The safety and quality of sous-vide products rely on good control and monitoring of hygiene during the entire chain of preparation, packaging, processing and distribution. Guidelines recommend heating to 70°C for a minimum of 2 minutes and then store and distribute a product at temperatures below 3°C, with a maximum shelf life of seven days. Because of the potential risk for growth and toxin formation by Clostridium botulinum, heat treatment to 90°C for 10 minutes or equivalent is recommended to permit a shelf life of more than 10 days, (Martens and Lucetti, 1999).

Packaging

Modified atmosphere

In order to prolong the shelf life, fresh meat and fish, prepared foods and baked foods are packed in modified atmosphere, with high concentrations of CO_2. The principle is the replacement of air in the package with a fixed gas mixture, composed of different levels of oxygen, nitrogen and carbon dioxide. When microbiological spoilage is the main parameter, high levels of CO_2 is used. CO_2 is the most important gas as it has bacteriostatic and fungistatic effects. The effectiveness is strongly depending on the storage temperature as the solubility of CO_2 on the food surfaces decrease rapidly with increasing temperatures. For red meat products oxygen must be present to maintain the red colour. For other products oxygen levels are kept low. Other gases such as Argon and carbon monoxide have also been investigated experimentally or used on a restricted commercial basis.

The practical application of MAP involves finding the correct gas mixture and the right balance of food to gas volume in the package. A sufficiently high concentration of CO_2 on the food surface is essential for the effect on micro-organisms. Furthermore the permeability of the packaging material to CO_2 is important to control.

MAP is applied to a large extent for prolonging the shelf life of chilled seafood products. For reaw fish extension in the range of 50-100% in storage life is reached and for cooked fish extension of 100-200%. For fatty fish oxygen should not be included in the gas mixture, whereas for lean fish and seafood, 20-30% oxygen in the gas mixture gives lower drip losses, (Sivertsvik et al, 2002).

Active packaging

This term covers packaging methods and agents that actively influence the shelf life of the food during storage. The best-known example is the oxygen absorbers or scavengers, which reduce the oxygen levels in the headspace and also absorbs permeating oxygen. The scavengers come as small sachets or tablets to be introduced into the package. The scavengers are today also incorporated into packaging films. Most of the currently used absorbers are based on Iron. The reduced level of oxygen in the headspace prevents development of rancidity, and is also effective in reducing growth of certain types of micro-organisms, e g moulds. Other active packaging systems rely on absorption of ethylene respiring fruits and vegetables. Another type of active packaging involves ethanol vapour generators. The ethanol absorbed on silicon dioxide powder and contained in paper sachets prevents the growth of moulds. They are mainly used for bakery products, (Rooney, 1995).

Edible coating

The rapid development of biodegradable films for food packaging has helped to strengthen the development of edible coating applied directly on the foods. Coatings are made from films of proteins, starches or waxes etc. The coating will add protection against oxygen and moisture migration to the product, reducing the requirements on packaging. Often the edible coatings also contain agents such as preservatives or reducing agents that also contribute to prolonging the shelf life. Most films are sensitive to moisture, which limits their application to dry, frozen and semi-moist foods.

Conclusion

The minimal processing technologies represent a means of meeting the well-established, long-term trends in consumer demands for convenience, variety and fresh-like quality. Such technology will allow the food industry the possibility of producing high-quality, high value added products to meet future consumer demands. The technologies require varying degrees of capital investment. But most importantly, the technologies require investment into product and process know-how, not only of the minimal-processing technology itself, but also of the integrated chain of food distribution from agricultural production to the consumer. Future research into minimal processing technologies must include integrated approaches involving many different aspects along the production chain, such as hygienic design and handling of products and packaging and the logistics of the distribution.

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L 5. USE OF CARBON MONOXIDE FOR COLOR RETENTION IN FISH

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Abstract

The use of carbon monoxide (CO) applied either as a single gas or a component in 'tasteless smoke' (TS) is increasing in both domestic and international fish commerce in the United States. Following initial investigations in the early 1960's with modified atmospheric packaging and the traditional knowledge of fish smoking, commercial interest submitted petitions for regulatory recognition of this practice in 1999. A controversial decision by the U.S. Food and Drug Administration suggested recognition as a generally recognized as safe (GRAS) procedure with required labeling to distinguish treated products. This controversy continues and has extended to recent approvals for red meats. This situation parallels similar controversy in Europe where certain nations desire continued use of CO for meats (Norway), yet the EU does not currently recognize use of CO treatments for seafood.

Commercial interests are divided. While there is a definite growing market for CO treated products based on product appeal and convenience, processors debate due to concerns for preserving, enhancing, and masking of inferior products. This is countered by support for a processing method that now allows use of freezing which can reduce potential product quality and safety problems that are more common in fresh fish commerce, i.e., discoloration and elevated histamines, respectively. A primary concern is increasing competition from frozen products that can retain colors vs. traditional fresh products with natural colors denoting freshness and value per color grades. Likewise, for certain histamine-prone species, color retention could mask potential scombrotoxic products, and some adverse opinions question the safety of CO treated foods.

Science is rapidly evolving to better direct regulatory and commercial decisions. Although recent human feeding trials have demonstrated that CO treated fish do not pose a health threat, elevated histamine levels are possible in certain thermally abused CO treated species that are otherwise apparently fresh. Commercial applications have expanded beyond the initial applications with tunas to numerous species from both wild and cultured sources. Abusive use both in terms of discolored product and enhancing inferior product is possible. Attempts to develop measures to detect CO treatments have been questionable for both the quantity of residual gases in tissue or the utility of color standards. Current work is focusing on examining the effect CO and TS has on various quality and safety factors of different fish species as well as elucidating what occurs on the molecular level with different fish muscle components. This work intends to develop rapid and improved methods for CO detection in fish muscle.

Commercial Development

Processing methods to influence and retain color in fish muscle have been used probable since the first cavemen placed a piece of fish near a smoking fire. Although they had no understanding for the cause of the color change, product appeal encouraged continued use. Eventually the smoking techniques evolved to applications of cold smoke as a culinary art to impart colors without fully cooking the muscle. With the introduction of reduced oxygen packaging (ROP) and modified atmospheric packaging (MAP) during the 1960's, it became more obvious that product exposure to gas blends with carbon monoxide (CO) yielded favorable color development and retention. Patents began to appear in the late 1970's for specific applications of CO to impart color in meats, poultry and fish (Woodruff et al. 1985). Use of filtered smoke to concentrate the favorable components and CO were patented in the 1990's (Yamaoka et al. 1996). The most meaningful patent, in terms of fish applications, was issued to Kowalski (1999) for use of 'tasteless smoke' for color retention in frozen seafood. The success of this seafood patent encouraged more recent issues for CO applications to red meats, including ground beef (Shaklai, 2001).

Today CO is being applied to variety of seafood as a single gas with variable concentrations depending on product type and thickness, as a component in filtered or 'tasteless' smoke, and more recently as so-called artificial-filtered smoke based on gas blends to exemplify 'tasteless smoke'. Commercial use is expanding primarily with fish from either traditional harvests or culture operations in most seafood producing nation's about the world. The primary market driving this trend is based in the United States due to particular market acceptance, regulatory allowances, and the necessities for frozen products. The modes for application include 1) passive introduction in gassed enclosures or packaging, 2) active additions with pressurized chambers, and 3) post-harvest euthanasia of cultured fish. Despite some commercial efforts to develop processing and product standards through licensing and trade associations, market competition is attracting less experienced and less controlled applications. Although there are no obvious objections in the retail sectors, some wholesale competition with certain fresh fish is mounting efforts to eliminate these frozen CO entries or require sterner labeling. Market demand appears to be increasing with no objections to the atypical, or in some cases, 'plastic' colorations in certain fish, but public

opinion could be alerted by pending publicity to question the concept of 'CO treated' food. In the near future, public response could influence commercial practice and regulatory status.

Regulatory Status

In accordance with previous and current federal regulations in the United States, CO is an unapproved food additive [CFR Sec. 409 (a)(2)(C)], and use of CO to impart color in fish or other foods has been questioned relative to concerns for adulteration ['making a product appear better or of greater value than it is' – CFR Sec. 402(b)(4)] and deception through use of unapproved color additives [21 CFR Sec. 71.22]. Despite these regulations, the U.S. Food & Drug Administration (FDA, 2000-March) issued no objections to the GRAS petition ('generally recognized as safe' status) submitted by Hawaii International for application of 'tasteless smoke' in the preservation (color retention) of frozen tuna. FDA did not grant GRAS status. Their controversial response did emphasis specific labeling requirements for this 'preservative' and only addressed use with frozen tuna. Likewise, they included cautions of concern for enhancing the appearance of inferior products or potentially scombrotoxic fish.

Interestingly, in a related action, FDA (2002) published a similar response to another commercial petition for GRAS status for CO applications in MAP for fresh cut and ground red meats destine for retail display. Again, FDA did not object in any manner to limit use of the process while they did not grant GRAS status. Regulatory allowance for CO applications for color development and retention in muscle foods is becoming more apparent.

FDA considerations for use of CO or some form of tasteless smoke is influenced, in part, by prior federal regulations, which allow the use of smoke ('combustion products') in food processing with some specific citations for exposure concentrations of 4.5% by volume (21 CFR 173.345). Smoking is a recognized form of seafood processing with specific HACCP requirements. Likewise, the components of smoke, which include CO, impart color development and retention irrespective of the treatment temperature. The color development in traditional smoked seafood could enhance and mask product concerns for food quality and safety no more so than errant applications of CO/tasteless smoke for frozen products. It must also be recognized that the use of CO/tasteless smoke to allow freezing of fish, that would otherwise be shipped fresh to avoid detrimental color changes that occur when certain fish species are frozen, provides a significant reduction in potential histamine/scombrotoxin hazards.

Concurrently, the voluntary inspection program, based on fees-for-service conducted by the National Marine Fisheries Service (USDC/NMFS, 2003), has been working with various international firms to provide process verifications and operation audits for CO/tasteless smoked products to be imported into the United States. In addition to their prerequisites for sanitation and product quality, NMFS has tried to develop a color scheme that relies on actual measurements for color shades and progressive fading in thawed product that distinguishes the proper commercial methods. They provide a listing of the approved firms verified under contract with NMFS.

Presently, similar CO /tasteless smoked fish products are not approved for commerce in countries other than the United States. Japan issued a notice banning fish that have an initial CO content $\geq 500\mu$g/kg, or an initial content $\geq 200\ \mu$g/kg which decreases significantly during two days refrigerated storage. (Japan, Food Sanitation Law-Article 6). Reasoning for these limits is not clear and analytical governance is subject to significant error such that treated product is indeed sold in Japanese markets. Similarly, Canada does not recognize or approve use of CO treatments for fresh or frozen fish, but use of tasteless smoke has remained in consideration since 1999 (Prince, 1999 and Andruczyk, 2001). Although it appears as if the EU still objects to the use of CO treatments as a food additive for any food, recognized use with fresh meats in Norway has stirred concern for further considerations (EC, 2001).

Science

As with the cavemen, scientific understand for the specific applications of gases with CO to impart colors in frozen fish is somewhat lacking. The knowledge of heme protein reduction and oxidation must be applied with certain fish with considerations for the commercial situations and market consequences. Fortunately, initial work in human feeding trials with CO treated fish have substantiated the initial calculations that CO treated seafood do not pose a health threat to consumers (Davenport, 2000). Ross (2000) introduced a standard color scale to monitor consequence of CO applications at 4% and 100% to yellowfin tuna (*Thunnus albacares*). Her work indicated the depth of color development and duration of color retention was influenced by prior CO concentration and length of product exposure. Sensory ratings, total plate counts and measurements for progressive development of cadaverine and histamine levels suggested the CO treated tuna was less problematic than the same tuna without prior CO exposure. Lab trails initiated by Balaban et al. (2003) in 1999 indicated CO applications with variable concentrations and exposure times yielded similar results for tuna, cultured catfish and tilapia. Kristinsson et al (2003) are actively investigating the influence of CO applications with variables for fish species, gas concentrations, and product quality prior to and after CO exposure. This work ranges from applied applications to investigations at the molecular level to assess potential controls for commercial and regulatory use.

Remaining questions that need research attention:

1. Can inferior fish quality, as denoted by development or loss of particular meat colors, be reversed by CO applications that enhance product appearance? What degree of transition is possible and does this involve potential food safety concerns?

2. Can controls be developed to monitor CO applications to suit commercial and regulatory guidelines for product quality and safety? Would these controls vary by fish species, product types (fillets vs. whole fish) and product forms (fresh vs. frozen)?

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L 6. THE EFFECT OF CARBON MONOXIDE AND FILTERED SMOKE ON THE PROPERTIES OF AQUATIC MUSCLE AND SELECTED MUSCLE COMPONENTS

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Abstract

Carbon monoxide (CO) and filtered wood smoke (FS) rich in CO have been used to stabilize fish muscle color. This stabilization comes from the high-affinity binding of CO to heme proteins leading to a cherry red appearance of dark muscle. There is a great lack of information on what occurs with fish muscle and muscle components after treatment with CO or FS.

Our interest was therefore to investigate: (A) how CO affects the molecular properties of hemoglobin (one of the main molecule behind the color stabilization), and (B) how different CO and FS treatments affected the quality and safety of fish muscles from different species.

(A) Tilapia hemoglobin (Hb) isolated from blood was used as a model Hb. Different derivatives of Hb were subjected to different denaturation experiments (thermal, pH and chemical denaturation). Hemoglobin autoxidation and denaturation on storage at different temperatures and pH's was determined and hemoglobins role as a catalyst of lipid oxidation examined on fish phospholipids. (B) Mahi mahi and Yellowfin tuna muscles were treated for 48 h at 4C in different CO/FS environments and color (using a digital color machine vision system), lipid oxidation (TBARS), heme oxidation state and histamine formation determined during frozen (-30C) and refrigerated storage (4C).

Results show that carbon monoxide significantly stabilized tilapia hemoglobin in terms of denaturation. CO-Hb also was significantly more stable towards autoxidation (and thus color changes) and had decreased pro-oxidative activity. Fish treated with CO and FS had significantly improved color on treatment and color stability during storage. Using digital machine vision analysis a detailed color analysis could be achieved identifying representative color and color changes for each treatment. This was directly related to the heme proteins stability which was enhanced on CO/FS treatment. Pure CO had a more dramatic effect on color compared to FS. Lipid oxidation was reduced for some of the CO/FS treatments while the treatments had however little effect on histamine formation which could be of concern.

Introduction

The challenge facing commercial fisheries is to maintain color characteristics of aquatic foods during processing, transport, storage and display and at the same time assure safety (Ross, 2000). One of the main product attributes of dark muscle fish species is their bright red color arising primarily from the oxygenated and reduced form of the heme proteins myoglobin and hemoglobin (Mb/Hb-Fe^{2+}-O_2). Dark muscle is however very susceptible to discoloration after it is cut and also on freezing, yielding a brown color due to the oxidation of the heme proteins to give metHb and metMb (Mb/Hb-Fe^{3+}) (Livingston and Brown, 1981). This discoloration is highly undesirably as the product becomes less appealing to the consumer, and thus leads to a lower price than a bright colored cut. To avert discoloration some processors/importers introduced the use of carbon monoxide (CO) and filtered smoke (FS) containing CO to stabilize red muscle color prior to freezing. This stabilization is due to the strong biding of CO to the heme in hemoglobin and myoglobin (Mb/Hb-$Fe^{2+}\equiv$CO) (e.g. >240 times higher affinity in Mb compared to O_2), making it highly resistant to autoxidation, and thus discoloration (Stryer, 1988; Sorheim et al., 1997). These processes have stirred considerable controversy since many argue that the process represents economic fraud and that CO/FS treated products could mask underlying seafood safety problems (growth of pathogens and formations of biotoxins). On the other hand there are also obvious benefits of this process to the consumer as well as the industry. With proper controls for CO/FS treatments, and product identity and more scientific data on the processes this new practice can maintain product appeal and allow for other processes, e.g. freezing, high-pressure, irradiation as a significant control to reduce potential incidence of pathogens and biotoxins. This will ultimately be of benefit to the consumer, in terms of product availability, price, safety and quality. This goal will however not be reached until a sound scientific understanding is obtained on these processes. Scientific data on the effect of CO/FS on product quality and safety is greatly lacking.

At the University of Florida there is an active research program aimed at investigating different basic and applied aspects of processing aquatic foods with CO and FS. Our aim is to understand how these processes affect individual components of fish muscle on the molecular level as well as investigating the impact of these processes on the macroscopic level, i.e. on aquatic food quality, acceptability and safety. Here we report on the effect carbon monoxide has on the properties of hemoglobin, one of the main protein responsible for color preservation after CO and FS treatment, along with data on the effect of CO and FS treatment on select quality attributes of two different

fish species, mahi mahi (*Coryphaena hippurus*) and yellowfin tuna (*Thunnus albacares*), both of significant commercial value to the US.

Materials and Methods

A) Effect of carbon monoxide on the properties of hemoglobin. Hemoglobin (Hb) was isolated from blood of tilapia according to Rowley (1990) and was used as a model Hb. Different derivatives of Hb were prepared (CO-Hb, Oxy-Hb and met-Hb). CO-Hb was compared to Oxy and met-Hb for its stability to denaturation and oxidation. Isothermal thermal denaturation experiments were performed from 45-70°C and protein and heme denaturation followed spectrophotometrically. pH denaturation experiments were performed from pH 1.5-12 and changes in protein conformation followed from changes in the Hb UV-visible spectra and tryptophan fluorescence according to Kristinsson (2002). Chemical denaturation in 0-6M guanidine-hydrochloride and urea were performed and conformational changes monitored as described above. Autoxidation of Hb as a function of different temperatures (-30°C, 4°C and 20°) and pH's (pH 6-8) was studied using UV-visible spectroscopy. The ability of Hb to function as a lipid oxidation catalyst was explored in a washed fish muscle system following the formation of thiobarbituric reactive substances (TBARS) (Richards and Hultin, 2000).

B) The effect of carbon monoxide and filtered smoke on the chemical and microbial quality of fish muscle. Mahi mahi and Yellowfin tuna were obtained fresh within 3 days of harvest. Mahi mahi was filleted and skinned while loins were cut from the tuna. Fillets and loins where aseptically cut into several pieces and treated for 48 h at 4°C in different gas mixtures; 4%CO, 20% CO, 100% CO and FS (which contains 20% CO). Samples were then vacuum packed and frozen at -30°C for 1 month, followed by thawing and refrigerated storage at 4°C for 8 days. In a separate experiment samples were left in the gas environments at 4°C for up to 14 days at 4°C. Samples were analyzed before and after treatment, after frozen storage and on refrigerated storage for: (a) Color, using a digital color machine vision system (Balaban et al., 2003) and a Minolta hand-held colorimeter, (b) lipid oxidation products, using TBARS (Lemon, 1975), heme protein oxidation state (Kristinsson, unpublished data), aerobic microbial growth, using Petri-films incubated at 25°C for 48 h (AOAC, 1995), and histamine formation followed with a ELISA-based Veratox ® Histamine analysis method (Neogen Corp., Lansing, MI). CO/FS treated fish were also subjected to sensory evaluation.

Results and Discussion

A) Effect of carbon monoxide on the properties of hemoglobin

When complexed with carbon monoxide hemoglobin was substantially stabilized compare to oxy-Hb and met-Hb (which was the least stable). CO-Hb exhibited greatly increased stability towards thermal denaturation, was able to largely retain its structure down to pH 3.5 and up to pH 12 and was more resistant to chemical denaturation. UV- visible spectroscopy results show that this stabilization comes from the ability of CO to stabilize the heme environment in Hb. CO-Hb did also have substantially higher oxidative stability (i.e. resisted the formation of met-Hb) and thus color stability compared to oxy-Hb at all temperatures and pH's tested. This oxidative stability was greatly enhanced at pH 8 vs. pH 7 and 6 also was significantly more stable towards autoxidation due to an enhancement in the binding of CO in the heme pocket of Hb. Complexing Hb with CO also lessened its ability to oxidize fish membrane lipids, thus serving an anti-oxidative purpose. This lower pro-oxidative ability is likely due to increased stability towards autoxidation of the CO-Hb compared to oxy-Hb.

B) The effect of carbon monoxide and filtered smoke on the chemical and microbial quality of fish muscle.

Fish treated with CO and FS had significantly enhanced red color (a-values) on treatment, which was favored by sensory panelists over untreated control. Gas treatment led to significant color stabilization on freezing, thawing and cold storage compared to untreated controls. Color enhancement and stability was directly proportional to the percent CO used to treat the fish and subsequently the stability of heme proteins after treatment as assessed by Hb's UV-visible spectra. Employing digital machine vision analysis it was possible to obtain a detailed color analysis and color stability kinetic analysis by identifying classes of colors and hues representative of each treatment. Lipid oxidation was reduced for the CO/FS treatments except for the 4% CO treatment. The increased oxidative stability correlated with increased stability of the heme proteins in the muscle to autoxidation. CO and FS treatment lowered aerobic microbial counts and extended microbial shelf life of both mahi mahi and tuna, likely as a result of reduction in oxygen. CO/FS treated samples developed histamine both during gas treatment and on storage, however histamine developed slower as percent CO increased, indicating an effect on histidine forming microorganisms.

Conclusion

Carbon monoxide had a significant stabilizing effect on fish hemoglobin, which translated to increased resistance towards hemoglobin denaturation, autoxidation (and thus color change), and a lower pro-oxidative activity. These results suggest that quality problems arising from heme proteins in aquatic foods are likely to be lessened by treatment with CO or FS. Studies with muscles from mahi mahi and yellowfin tuna confirmed this as significant stabilization of color and oxidation resulted from CO and FS treatment, as well as extended microbial shelf life. The results show that responsible use of CO and FS can have positive effect on product quality and safety.

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L 7. THE INFLUENCE OF HIGH HYDROSTATIC PRESSURE ON THE WATER HOLDING CAPACITY OF FISH MUSCLE.

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Abstract

During frozen storage of certain species of fish changes occur which result in deterioration of the textural attributes of the cooked fillet. The observed textural changes are characterised by an increase in firmness of the fish, concomitant with a greater propensity for water to be lost on thawing and/or cooking. The problem is most acute for the white flaky fish fillets of, for example, cod haddock and hake. Generally it is reported that the textural changes occurring during frozen storage are due, ultimately, to changes in the myofibrils.

Our current hypothesis is that changes in the water holding capacity of fish fillets on frozen storage are due to changes in myofibrillar water holding capacity, and that these changes are due to association of the myofibrillar thick filaments. This paper suggests how selective denaturation of domains within the myosin molecule, the main constituent protein of the thick filaments, by ultra high pressure may be used to control the water holding capacity of fish fillet through freezing and frozen storage.

The partial denaturation of myosin by ultra high pressure was monitored by differential scanning calorimetry (DSC). The effect of pressure on the myofibrillar ultra-structure has been investigated by transmission electron microscopy. The changes in the water holding capacity have been investigated by pulsed proton nuclear magnetic resonance (NMR). The results from these techniques will be discussed.

Observations indicated that the application of a pressure of 100 Mpa, to the cod fillet prior to freezing, caused denaturation of parts of the myosin molecule and slight changes in the ultra structure of the myofibrils. These subtle changes also led to a reduction in cook loss. However, as the pressure was increased to 200 MPa denaturation of actin was observed, and this was coincident with a dramatic disruption of the myofibril structure. Data suggest, therefore, that denaturing the thin filament protein actin caused a reduction in the water holding capacity of the myofibrils. The results presented indicate that by careful selection of pressurising conditions the water holding capacity of fish filets can be manipulated, and these changes in water holding capacity are maintained through freezing and frozen storage.

Introduction

During frozen storage of certain species of fish changes occur which result in deterioration of the textural attributes of the cooked fillet. The observed textural changes are characterised by an increase in firmness of the fish, concomitant with a greater propensity for water to be lost on thawing and/or cooking. The problem is most acute for white flaky fish, such as cod, haddock and hake[1,2,3,4].

It has been shown that the myofibrils occupy a substantial proportion of the volume of muscle, and that changes in water holding capacity of whole muscle arise from changes in the water holding capacity of the myofibrils[5]. Therefore, it is likely that the observed changes in the water holding capacity of frozen fish fillet are due to changes in myofibrillar water holding capacity. Consistent with this idea, it is generally reported that the textural changes occurring during frozen storage are due, ultimately, to changes in the myofibrils[6, 7, 8] . Our current hypothesis is, therefore, that changes in the water holding capacity of fish fillets on frozen storage are due to changes in myofibrillar water holding capacity, and that these changes are due to association of the myofibrillar thick filaments.

The main constituent protein of the thick filament is myosin. The myosin molecules are organised in such a way so as to allow the myosin head regions to protrude from the main body of the thick filament. This allows them to interact with the thin filaments (whose main constituent protein is actin) during muscle contraction when the fish is alive. If inter-thick filament associations occur in the frozen state, it is most likely that they are via myosin head associations. These may be either association of myosin heads with myosin heads on neighbouring thick filaments, or via the association of myosin heads with another part of a neighbouring thick filament. Therefore, it may be hypothesised that selective denaturation of the head domains would reduce the number of inter-thick filament interactions occurring during freezing and frozen storage. Hence, this could be used to control the water holding capacity of fish fillet. The next paragraph highlights the principles of ultra-high pressure and how pressure may be used to selectively denature the myosin head regions.

A system at equilibrium will tend to minimise the effects of an external perturbant (Le Chatelier's Principle). Application of pressure will, therefore, favour changes that result in a reduction in volume. Consequently, high hydrostatic pressure tends to stabilise hydrogen bonds since their formation results in a slight reduction in volume.

Conversely, ionic bonds are broken since their disruption results in a decrease in volume. Similarly, hydrophobic bonds may also be disrupted at low pressures, whilst covalent bonds tend not to be affected by high hydrostatic pressure.

The myosin heads are stabilised by internal hydrophobic bonds and will tend to be denatured under ultra high hydrostatic pressure. Conversely, the myosin rod is stabilised, predominantly, by hydrogen bonding, and is likely to be stabilised by ultra high pressure. It is possible, therefore, to selectively denature the myosin head region without inducing large changes in the main body of the thick filament[9, 10], thus preserving most of the structural integrity of the myofibrils.

The thin filaments contain the protein actin. The actin molecules are globular in nature, and are joined together rather like two strings of pearls twisted together to form the thin filaments. The actin molecules are stabilised by hydrophobic bonds, and will also be more likely to be denatured by high pressure[10].

By application of ultra high hydrostatic pressure it may possible to denature selectively the myosin molecule. By selectively denaturing the myosin head regions, so rendering them "inert" prior to freezing, it ought to be possible to prevent or at least reduce thick filament association whilst frozen. Reducing the extent of thick filament association in the frozen state will allow the myofibril to recover its original volume on thawing. This will in turn reduce water losses from the fillet. In addition, by denaturing neither the helical regions of myosin nor the actin in the thin filaments, the myofibril structure will be largely preserved. Consequently, the fish will have a raw and unprocessed appearance.

A technique that may be employed to report upon the water location changes in muscle is pulsed proton nuclear magnetic resonance (NMR) [11, 12]. The spin-spin or T_2 relaxation time of water protons is dependent upon the motional correlation time of the water molecule. The motional correlation time reflects the overall isotropic reorientation, and can be defined, as the average time required for the water molecule to rotate by one radian. Thus, a water molecule experiencing restricted motion at, for example, the surface of rigid protein structures within a myofibril, will have a shorter spin-spin relaxation time than water molecules that are unable to contact the surface. Thus, a distribution of relaxation times may be observed, which reflects the extent to which water molecules interact with the myofibrillar structure[11], and consequently will relate to water holding capacity.

It has been shown that the spin-spin relaxation times of water in whole muscle are complex[13], but reflect the spacial heterogeneities that exist within the structure. Also, is has been shown that changes in the distribution of spin-spin relaxation times reflect changes in the ultra-structure, water holding capacity and sensory properties of meat and fish[14, 15, 16]. Thus, the NMR relaxation technique is a useful tool in probing the water distribution changes associated with frozen deterioration of fish texture, and may also report on changes in sensory attributes relating to water distribution changes.

The thermal unfolding of myosin is complex[17], and has been interpreted in terms of the co-operative unfolding of different domains within the myosin molecule. For example, unfolding of the myosin head regions could occur, independently of and at a different temperature to the unfolding of myosin rod domains. Therefore, the partial denaturation of myosin by ultra high pressure was monitored by differential scanning calorimetry (DSC).

Materials and Methods

Fresh cod fillets were obtained from a local supplier of fresh fish. The freshness of the fillet was assessed by K value determination and the period of ice storage prior to purchasing was estimated to be 1-2 days. The pHs of the fillets were also checked. Samples of the fish fillets were minced and homogenised with de-ionised water to make a 10% suspension (1g of fish in 9g water). The pHs of all the cod samples used in this study were between 6.7 and 7.0,

The fillets were vacuum-sealed into bags and the sealed bags placed in plastic bottles containing iced water. Iced water was used so as to prevent the sample temperature increasing above 10^{o}C during the pressurisation. The pressures were applied for 20 minutes once the selected pressure had been achieved.

Cook-looses were determined for samples vacuum-sealed into bags and heated for 15 minutes at 80^{o}C in a water bath. The samples were rapidly cooled in cold water and the cool-loss determined.

The samples for frozen storage were blast frozen at -30^{o}C for two hours the equilibrated at -80^{o}C overnight. The samples were then stored at -12^{o}C.

The spin-spin proton relaxation times were determined in the following manner. Samples were loaded partially frozen into sealable glass tubes (circa 1 g per sample), and allowed to thaw. The spin-spin relaxation times (T_2) were measured using the CPMG pulse sequence on a Bruker CXP Spectrometer operating at 60MHz for protons. Measurements were made both before and after cooking (80°C for 15 minutes in a water bath). The samples were cooked in the NMR tubes. All measurements were made at 20°C.The acquisition parameters were:

180° pulse separation = 200μs
Delay time between pulse trains =10s
Number of scans per sample = 5

The water distribution profile was determined from the Fourier transform of the relation decay after the method of Clark and Lillford [11,18]

Differential scanning calorimetry was carried out using a Setaram micro DSC II. Samples of approximately 700mg were loaded into 1cm^3 stainless steel cells and scanned over the range 10°C to 90°C at a rate of 0.5°C / min against a water reference.

The transmission electron microscopy (TEM) was carried out in the following manner. Small pieces (1-2mm) of fish muscle were fixed in 2.5% glutaraldehyde in 0.1 M sodium cacodylate buffer pH 7.2 as soon as they were received. After initial fixation for 3 hours the samples were washed with several changes of buffer solution and stored overnight in the fridge. Post fixation was in 1% osmium tetroxide in 0.1 M sodium cacodylate buffer pH 7.2

for 2 hours. The samples were then block stained overnight in 1% aqueous uranyl acetate. Dehydration was via ascending grades of ethanol (70%, 90% and 100%), followed by 100% acetone, that acts as an ante-medium between the alcohols and the resin. Samples were then soaked in a 50/50 mixture of Araldite epoxy resin and acetone overnight, followed by immersion in pure resin for 24 hours. Finally the samples were embedded in plastic moulds and polymerised at 60 degrees C for 48 hours. Ultra-thin sections were cut on a Reichert Ultracut E ultra-microtome at 100 nm thick using a Diatome diamond knife. Sections were collected on 200 mesh copper grids and stained with Reynolds lead citrate. All the sections were examined and photographed using a Jeol 1200 EX transmission electron microscope operated at 80KV.

Results and Discussion

It has now been shown that the application of ultra high pressure can reduce cooking loss from frozen cod fillets. For example, it has been observed that application of a pressure of 100 MPa to the cod fillet (prior to freezing) caused a reduction in cook loss from around 25% loss in the untreated fillets to around 15% in the fillets treated at 100 MPa pressure. After application of 100Mpa pressure it was observed that denaturation of parts of the myosin molecule had occurred, along with a slight change in the ultra structure of the myofibril. However, as the pressure was increased to 200 MPa denaturation of actin was observed, and this was coincident with a dramatic change in the myofibril structure. However, some domains of the myosin molecule (assumed to helical regions) were not denatured at any of the pressures studied. Data suggest, therefore, that denaturing the thin filament protein actin caused a dramatic change in the myofibrillar structure and a decrease in the water holding capacity of the fish fillet. Thus, it is clear that by careful selection of pressurising conditions a suite of fairly specific changes can be induced. This is an important observation, and should be born in mind when selecting pressurisation treatments to, for example, improve microbiological and shelf-life stability of fish products.

NMR data also suggest that these increases in water holding capacity can be maintained through freezing and frozen storage. It is the aim of future studies to investigate the impact that high hydrostatic processing has on the sensory properties of cod fillet, and to determine if the textural quality of pressure treated cod is preserved during frozen storage.

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L 8. HIGH PRESSURE ASSISTED THAWING OF FROZEN FISH FILLETS: IMPACT OF DIFFERENT PRESSURE LEVELS ON QUALITY ASPECTS

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Abstract

The use of high hydrostatic pressure enables controlling the freezing and melting point of a food product. Increasing pressure decreases the melting point of pure water to a temperature value of –22 °C at 210 MPa. Thus a high absolute temperature difference between product and surrounding medium can be carried out at moderate temperatures. Accordingly, the melting enthalpy of ice I decreases likewise to a minimum of 240 kJ/kg at the triple point (liquid/ice I/ice III). Consequently, the required time for phase transformation (solid-liquid) can be reduced significantly. Depending on the pressure level and the medium temperature applied, a phase transition time reduction of approx. 40% (100 MPa) to 60% (200 MPa) is achieved at medium temperatures of 10 °C when comparing to conventional thawing processes.

A comparison of the influence of pressure-supported thawing at 100, 150 and 200 MPa and thawing in water at ambient pressure on the quality of thawed fillets from rainbow trout and ocean perch has been carried out using sensory, physical and microbiological methods. Data showed that pressure assisted thawed fillets were visually distinguishable from those thawed in water irrespective of the pressure level applied. The lightness decreased with reduction of pressure. Organoleptically the raw high pressure treated fillets were evaluated better mainly for texture before cooking compared to conventional thawing in water. The cooked sample thawed at 100 MPa pressure was evaluated comparable to the conventionally thawed one. Increase of pressure led to quality alteration of cooked samples. Thaw drip was reduced with increasing pressure. DSC measurements revealed that thawing at both 200 and 150 MPa denature muscle proteins markedly. However, the samples thawed at 100 MPa were almost comparable to the conventionally thawed fillets. Further differences resulted in the total viable count, pH value, water binding ability, and in the instrumentally evaluated texture parameters.

Introduction

Thawing generally occurs more slowly than freezing. During thawing, foods are subjected to damage by chemical and physical changes and by microorganisms. Quick thawing at low temperature to avoid notable rising in temperature and excessive dehydration of food is desirable to assure food quality as pointed out by Li and Sun (2002). As outlined by them, some recent research revealed that high pressure thawing can preserve food quality and reduce the necessary thawing time, suggesting its potential for the food industry. Limitations on the application of high pressure thawing are mainly high cost, pressure-induced protein denaturation and meat discoloration. A reduction in thaw drip was observed by Chevalier and others (1999) and Rouille and others (2002), who mentioned additionally 150 MPa being best suited. It could be very recently shown (Schubring and others (2003)) that high pressure supported thawing at 200 MPa of fish fillet processed from various species markedly shortened the thawing time by about 50% compared to conventionally thawing in water at ambient pressure and temperature. At the same time, the thaw drip is significantly reduced and the high pressure thawed fillets increased significantly their lightness. Sensory parameters of both conventionally thawed and HP thawed fillets were at least comparable. However, some drawbacks were considered concerning the sensory quality of thawed-then-cooked fillets and other signs of protein denaturation, i.e. differential scanning calorimetric curves, increasing firmness and toughness and reduced water binding ability.

The object of the present work was to optimise the pressure used for thawing regarding the quality of the thawed and subsequently cooked fish fillets and the other shortcomings mentioned above noticed when 200 MPa was applied. Therefore, the fish fillets were thawed at 100, 150 and 200 MPa and compared to those thawed under atmospheric pressure at 10 °C.

Materials and Methods

The samples were processed as previously reported by Schubring and others, 2003. Fish species used included rainbow trout, Oncorhynchus mykiss, caught in a fish farm in Germany and ocean perch, Sebastes spp., caught during the 244th research trip of the FRV “Walther Herwig III” west of Greenland. Quality was evaluated by already reported sensory, physico-chemical and microbiological methods (Schubring and others, 2003).

Thawing process

In order to evaluate high pressure assisted thawing and the storing of foodstuffs, and to estimate the industrial potential of these processes, a transportable high pressure unit was used (Fig. 1). The pressure was built up using a high pressure reciprocate pump (DSXHW, Haskel Ltd., California, USA) which automatically compensates for pressure dependent density changes (volume changes) of the treated fish sample. The pressure transmitting medium (silicone oil) was chosen because of its suitable chemical and thermophysical properties within the relevant pressure and temperature range. A water bath (Haake 6P, Karlsruhe, Germany), equipped with a thermostat (Haake D1), was used to thaw the fish samples. The temperature of the circulating water was set to 10 ± 0.5 °C.

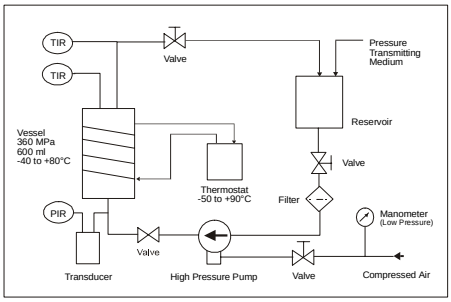

Fig. 1: Flow diagram of a high pressure apparatus for subzero (°C) operation

Typically thawing profiles are shown in Fig. 3. Compared to ambient pressure the phase transition time can be reduced significantly applying high pressure.

Results and Discussion

The sensory assessment using QIM adapted for the two species studied (Table 1 and 2) revealed that in thawed rainbow trout the following effects of high pressure were present: texture improved from slightly soft, plastic to firm, elastic, while the typical salmon-like colour got lost by turning into ivory; the melon-like odour turns into neutral, slightly sour odour. For thawed-then-cooked rainbow trout it can be noticed that under high pressure the odour and taste became slightly earthy and musty, while colour changed from bright-orange to greyish. On the other hand, the texture became dry, tough and stringy. When ocean perch was thawed by high pressure, the texture changed from plastic over elastic to plastic, odour turned from neutral, slightly hey-like to slightly faeces-like and colour changed from reddish-ivory to white. In thawed-then-cooked ocean perch the odour turned from neutral and slightly potato-like to slightly fishy; the colour changed from slightly grey to white and the taste became fishy, while the texture got less dry and tough. It can be summarised that not all changes by high pressure were unwanted. However, those regarding taste and odour and caused mainly by application of pressure higher than 100 MPa may limit other possible advantages from the technological point of view. Nevertheless, the effects of high pressure on fish samples vary with the species treated.

Table 1: Sensory assessment of raw and cooked fillet of rainbow trout

Thawed at (MPa)	raw				cooked			
	Texture	Odour	Colour	Gaping	Odour	Colour	Taste	Texture
0.1	1.3	0.3	0.3	0	1.2	1.2	2	1.3
100	0	0	1	0	1	2	2	2
150	0	1	2	0	1	3	2	3
200	0	1	1	0	1.5	1	2.5	2.5

Table 2: Sensory assessment of raw and cooked fillet of ocean perch

Thawed at (MPa)	raw				cooked			
	Texture	Odour	Colour	Gaping	Odour	Colour	Taste	Texture
0.1	0.7	0.1	1	0	0.1	0.7	0.3	1.6
100	0.3	0.3	0	0	1	0	1	1
150	0	1	0	0	0.7	0.7	1	0.7
200	0.7	1.3	0	0	0.7	0	1.7	1.3

The microbiological count was progressively reduced with increasing pressure applied to thaw fish fillet. A significant reduction in drip after thawing was already to be seen when 100 MPa was applied. A further reduction accompanied the increase of pressure to 150 and 200 MPa (Fig. 2).

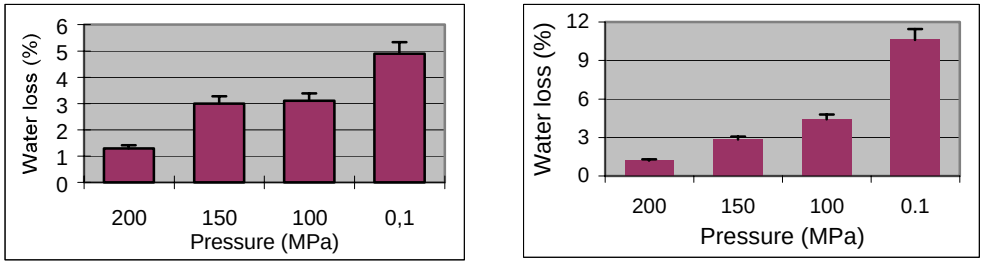

Fig. 2: Drip during thawing of rainbow trout (left) and ocean perch (right) fillet

However, the expressible moisture was lower after conventionally thawing compared to high pressure supported thawing. Instrumental texture measurement (firmness on intact fillet and penetration force on homogenates) did not show clear differences between the different treatments. Instrumental colour measurement, on the other hand, revealed pronounced differences especially in L* and ΔE* between conventionally and high pressure thawed samples, with L* being increased in the latter case. When protein denaturation was measured by DSC it became clear that high pressure curves are increasingly different from 100 to 200 MPa compared to that of conventionally thawed. Application of 100 MPa results in significant reduction of the phase transition time (Fig. 3), while the quality of the treated product remains comparable to conventionally thawed samples.

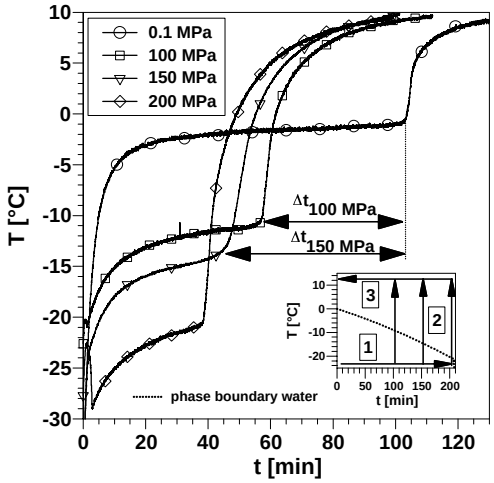

Fig. 3: Typical temperature evolution (sample center) during pressure assisted thawing at different pressure levels. The reduction of phase transition time is indicated by Δt. Insert: The processing steps are indicated (arrows) on the pt-diagram of water. 1: Pressure built-up; 2: Heating step and phase transition; 3: Pressure release.

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L 9. RADIO-FREQUENCY HEATING OF MINIMALLY PROCESSED FISH PRODUCTS - POSSIBLE QUALITY IMPROVEMENTS BY RAPID HEATING

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Abstract / Introduction

Heating of vacuum packed foods by a novel radio-frequency (RF) water bath process bears the potential of significant quality improvements for minimal processing of convenience cook/chill or cook/freeze fish products compared to conventional methods such as water baths, convection ovens and autoclaves. The rapid volume heating of the process is expected to reduce overcooking of the heat sensitive fish flesh and to result in a better product quality while maintaining product safety and shelf life. A heating curve is shown in figure 2.

An experimental RF-heater has been constructed which offers great flexibility in investigating and optimising the novel heating process. First RF-heating experiments have been carried out and demonstrated the feasibility of the process. It seems possible to reduce the heating time of the products by one order of magnitude compared to the heating time needed for conventional heating. Figure 1 shows a scheme of the RF-heating process.

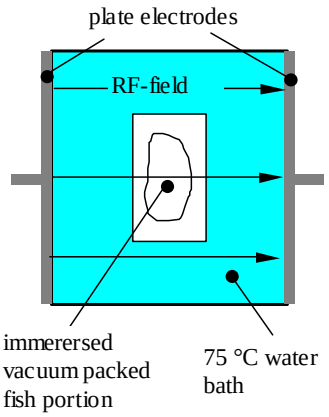

Figure 1: Scheme of RF-heater

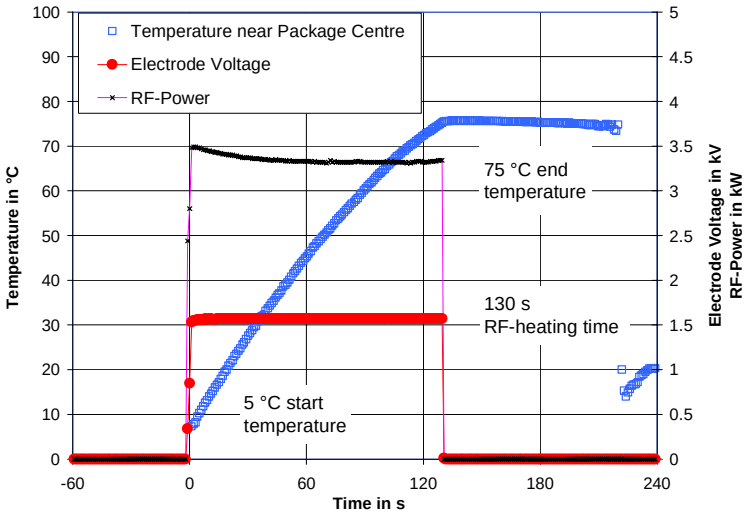

Figure 2: Heating curve of vacuum packed cod portion in RF-water-bath-heater

In order to get first verifications of quality improvements by rapid heating, experiments with microwave- and RF-heating of fish samples have been performed and are presented.

Material and Methods

The first experiments on the effects of rapid volume heating were performed with small samples from fresh Atlantic salmon fillets (*Salmo salar*) and fresh cod fillets (G*adus Morhua*) in a microwave oven. Reference samples were heated in an air convection oven. End temperatures for the heating were 75 °C, 95 °C, and 100 °C. Holding times were calculated using CTemp software (CCFRA, UK) to achieve the same cook values with both heating methods at all end temperatures. In the case of microwave heating the come up time to end temperature was 15 s to 35 s, the holding times at end temperature were 12 min to 1 min. The holding times in the convection oven varied from 27 min for 75 °C to 10 min for 100 °C.

A second experiment was performed in the experimental RF-water-bath using fresh, vacuum packed cod portions (140 g). An autoclave was used as the reference heater. The end temperature of the heating experiment was 75 °C and holding times were calculated to achieve the same pasteurisation value for RF-heating as for autoclave heating. Diagram 1 shows a typical heating curve of RF-heating, measured with a fibre-optical

temperature sensor at the core of the vacuum packed fish portion. Come up time to 75 °C for RF-heating was adjusted by power control to about 120 s, the holding time was 60 s. In the autoclave, the holding time was 16 minutes to achieve a similar pasteurisation value at the core.

To judge and compare the quality, all samples were analysed with respect to water holding capacity (WHC), cook-out, texture (force at 70% compression), and colour. In the second experiment, sensory evaluation was done using Quantitative Descriptive Analysis (QDA method) and Torry freshness score sheet.

Results

WHC /Cook-out

Results from WHC measurements of salmon (first experiment) and cod (second experiment) are presented in figure 3. All MW-heated salmon samples showed a significantly better WHC than convection-heated samples. The improvement in WHC by rapid heating is supported by a slightly lower cook-out of MW-heated salmon samples, which however was not very significant. RF-heated cod samples showed better cook-out, which was reduced to 21 % from a 26 % value in the case of conventional heating. WHC of RF-heated show an opposite effect for the cod samples shown in figure 3. MW-heated cod samples showed no significant difference between the heating methods.

Texture

Rapid heating by MW and RF seemed to have opposite effects on texture of the two fish species tested.

Microwave heated salmon was significantly firmer than conventionally heated samples. At 70% compression the mean force was 27% higher in MW-samples.

Compression tests on small sample cylinders of cod delivered lower mean values of compression force for all microwave- and RF-heated samples compared to conventionally heated samples. The difference was in the order of 20 % to 30 % and indicates a softer structure of rapid heated cod samples. The spread of the measurements was high and therefore significance of the results is not very firm. There was, however, a significant difference between MW and conventionally heated cod.

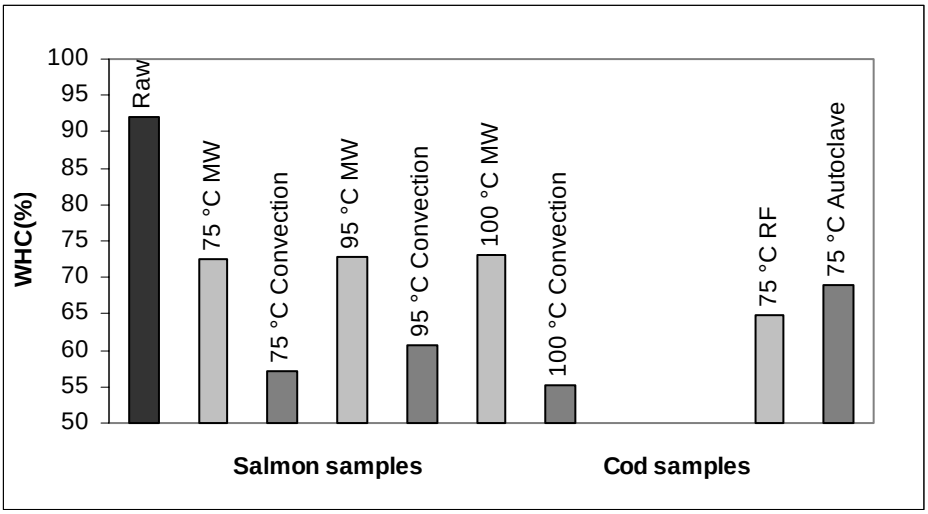

Figure 3: WHC in samples rapidly and conventionally heated.

Sensory evaluation

Results from the sensory evaluation are shown in Table 1. The RF-heated samples were more juicy and tender compared to conventionally heated samples. These results are in good agreement with the observation of improved cook-out and lower compression force in RF-heated cod. The scores for freshness were low compared to fresh cod.

Table 1. Mean scores of the cod samples. A mesh indicate a very significant difference to results with no mesh. QDA scores from 0 to 100%.

Heating method	Flakes	Firm-soft	Dry-juicy	Tough-tender
Conventional	63	42	32	41
Radio frequency	63	50	43	54

Conclusions

A significant improvement of water holding capacity of salmon (small samples) has been found when using rapid heating (microwave) instead of conventional heating (convection oven. It has been found that the thermal load on the edges of the conventionally heated samples is much larger than for rapid heated samples, thus giving a dryer product. A significant and repeatable difference between rapid heated samples and conventionally heated samples was found. Rapid heating by MW and RF seemed to have opposite effects on texture and WHC of the two fish species tested. A difference was found between RF-heated cod samples and conventionally heated samples with regard to juiciness and tenderness. This is a clear indication of the potential of the radio frequency heating technique.

However, it is suggested that this study is repeated. The raw material quality must be better monitored and the re-heating process might need more consideration. The scores of the sensory evaluation are low compared to fresh samples, and optimisation of the heating process is required.

Acknowledgement

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L 10. POSSIBILITIES OF NEW PROCESSING AND DISTRIBUTION OF SEA-FROZEN COD (*GADUS MORHUA*) FILLETS PACKAGED FROZEN UNDER MODIFIED ATMOSPHERE

(not to be quoted without prior reference to the authors)

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Abstract

The objective was to compare the effect of freezer storage on the keeping quality and storage life of thawed sea-frozen cod fillets stored in air and under modified atmosphere (MA). Sea-frozen fillets were kept 6 weeks, 8 months and 15-18 months in freezer storage (–24°C), then packed, thawed and kept for up to 25 days (0.5-1°C). Microbiological, chemical and sensory changes were investigated during storage. MA packaging of sea-frozen fillets prolonged shelf life of the thawed fillets compared to air packed fillets for up to 7 days. With longer freezer storage, the development of *Photobacterium phosphoreum* was delayed and the formation of TMA was slower.

Introduction

In recent years there has been a steady increase in the export of fresh fillets by air from Iceland to Europe resulting in higher prices per kg fish rather than selling fish whole or frozen at sea or land. Transportation by sea could be a realistic option if considerable increase of storage life of chilled fish can be obtained. Packing sea-frozen fish portions into ready-to-cook packages, thawing the fish during transportation and marketing it abroad as chilled fish could add value to fish processing. Danish scientists have reported that the bacterium *Photobacterium phosphoreum* is the main spoilage organism in fish packed under MA (Dalgaard et al., 1995,1997). However this bacterium has been found to be very sensitive towards freezing and freezer storage (Guldager et al., 1998; Boknæs et al., 2000). It was therefore of interest to investigate the effect of freezer storage on shelf life of thawed, sea-frozen fillets packed in MA compared to air and kept at refrigerated temperature.

Materials and Methods

Sea-frozen cod fillets kept for 6 weeks, 8 months and 15-18 months at –24°C were packed under MA ($CO_2/O_2/N_2$: 60/10/30) and in air, thawed and stored at 0.5-1°C. Samples of thawed fillets were taken at 3-4 days interval for up to 25 days. Counts of specific spoilage organisms (SSO), trimethylamine (TMA), trimethylamine oxide (TMAO) and total volatile bases (TVB) content, pH as well as sensory changes were monitored during storage. Total psychrophilic counts were done on Long and Hammer´s (LH) agar and counts of H_2S-producing bacteria were done on Iron agar. Spread-plating was used for both media and plates were incubated at 15°C for 4-5 days. Rapid conductance method (Malthus) was used to estimate counts of *Photobacterium phosphoreum* (Pp) as described by Dalgaard et al. (1996). Sensory evaluation was performed by IFL´s trained sensory panel using the Torry freshness score sheet (Shewan et al., 1953) and Quantitative Descriptive Analysis (QDA) described by Stone and Sidel (1985) but modified as only odour, flavour and texture attributes were used. The samples were cooked at 95°C in a steam oven for 7 minutes before served to the panel.

Results and Discussion

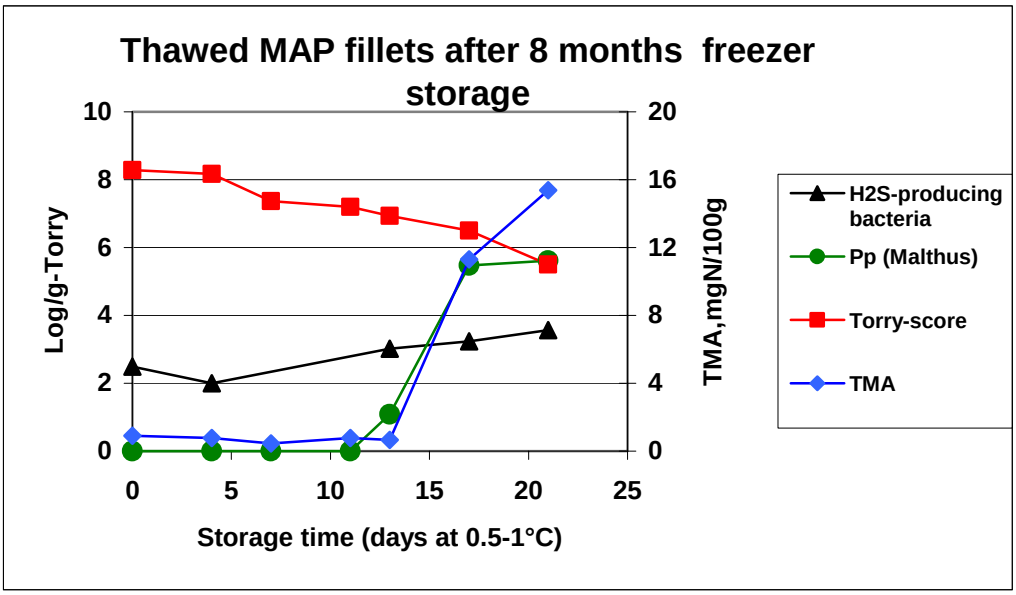
Freezer storage time did not apparently affect the microbial load of sea-frozen cod as similar total counts were found upon thawing. Similar growth curves were obtained for air-stored thawed cod, which was packed after 6 weeks and 8 months freezer storage, while a slightly slower growth was observed after 15 months. H_2S-producing bacteria were found in higher numbers after 8 months freezer storage than after 6 weeks storage. However, much lower levels were found after 15 months storage. *Photobacterium phosphoreum* (*Pp*) was not detected initially in thawed fillets after 6 weeks freezer storage but grew rapidly thereafter. After 8 months storage, delayed growth was observed after thawing followed by a rapid growth after 7 days of chilled storage. Similar findings were observed with thawed cod that had been stored for 18 months. After 6 weeks freezer storage, an increase in TMA and TVB was observed after 7 days of chilled storage in air. However after 8 months freezer storage, a similar increase did not occur until after 11 days in air storage. After 15 months freezer storage, very low levels of TMA and TVB were formed despite a high TMAO content.

MAP had a clear inhibitory effect on the microflora recovered following thawing and growth was further delayed with increasing freezer storage time. H_2S-producing bacteria were generally at low levels during MAP storage of thawed fillets from all experimental trials. Development of *Pp* was delayed under MA and influenced by freezer storage time, but growth occurred during late storage. After 18 months freezer storage, *Pp* was first detected at very low level (<1 log) towards the end of the storage period (21 days). After 6 weeks and 8 months

freezer storage, an increase in TMA and TVB was observed after 14 days of chilled storage in MA. However lower values were found after 8 months than 6 weeks. After 15 months freezer storage no increase in TMA and TVB was observed until after 21 days of chilled storage. This, in fact, probably corresponds to the low levels of *Pp* during chilled storage as observed after 18 months freezer storage. These results are in accordance with earlier results obtained at IFL in which it was concluded that the longer the freezer storage, the slower the formation of TMA (Magnússon and Martinsdóttir, 1995).

According to the results from sensory evaluation by the Torry-scheme, a prolonged storage life was observed in MAP fillets compared to those kept in air. The storage life of thawed fillets kept in air was estimated to 11 days after 6 weeks in freezer storage, but 14 days after 8 and 15 months freezer storage. The storage life of MAP fillets was estimated to 18 days after 6 weeks in freezer storage, but 21 and 25 days after 8 and 15 months freezer storage, respectively. However, very similar Torry scores were obtained for MAP and air fillets during the first 10 days of chilled storage, regardless of storage time in freezer. Shelf life of unfrozen fillets has been found to be up to 12 days on ice. (Magnússon and Martinsdóttir, 1995). MA-packing of unfrozen cod fillets extended the shelf life by 5 days (a part of this study not published here). According to sensory evaluation by QDA, the positive sensory attributes like characteristic sweet taste decreased during refrigerated storage. The negative sensory attributes, like freezer storage odour and flavour, sour odour and flavour, increased.

However the thawed fillets never received high scores for the positive attributes at the beginning of chilled storage after 15 months in freezer storage. Comparing the fillets kept in MA and air, the changes in general were very small for all sensory attributes during the whole refrigerated storage.



The MAP fillets were evaluated slightly drier and tougher than the air-stored fillets, especially after 15 months freezer storage. The average attributes for succulence and tenderness scored between 40 and 60 (100 points scale) for thawed fillets, which is in accordance to previous experiments (Martinsdóttir and Magnússon, 2001) but have been repeatedly found to range between 80 to 90 in unfrozen fresh cod by the panel at IFL (unpublished results).

The results from one of the storage experiments are shown on the above figure. Counts of H_2S-producing bacteria and *Photobacterium phosphoreum*, TMA content and freshness score (Torry) are shown for sea-frozen cod fillets that had been MA-packed after 8 months storage in freezer prior to thawing. At the end of the storage time (21 days) when the fillets had reached the limit of rejection (Torry score of 5.5), the total psychrophilic counts had reached log 7.1/g, while the level of *Pp* was close to log 6/g and the number of H_2S-producing bacteria was log 3.6/g. This difference in counts can be explained by the rather high tolerance of *Pp* towards CO_2. This bacterium is a very active TMA producer and can be assumed to be responsible for the TMA increase observed after day 13, concurring with its rapid growth.

Conclusion

MA-packaging of sea-frozen fillets prolonged shelf life of the thawed fillets (kept at 0.5-1°C) by 7 days compared to air-packed fillets. The option of using sea-frozen fillets to be retail-packed under MA before transport is an interesting alternative as it provides a shelf life of around 3 weeks. A constant and even supply of sea-frozen raw material for the chilled market of MA-retail packages is undoubtedly a great advantage. Based on the resulting shelf life, transportation by sea is a realistic option and could result in increased marketing abroad of chilled sea-frozen fillets.

These results are a part of a project funded by the Icelandic Research Council (RANNÍS) and the Icelandic companies ÚA and ÍSAGA.

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L 11. OPTIMISING GAS MIXTURES FOR MODIFIED ATMOSPHERE PACKAGING OF FRESH KING SALMON (*ONCORHYNCHUS TSHAWYTSCHA*)

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Introduction

Many researchers have evaluated the effect of modified atmosphere packaging (MAP) on the shelf-life of fish, and various gas mixtures have been recommended. However, published material does not provide a strong scientific basis for these recommendations (Bremer and Fletcher 1999). Studies have examined the effects of particular gas mixes on particular fish species, but their results cannot be used to predict the effect of different gas mixes on fish. Further, Devlieghere and others (1998) have demonstrated that the fish:gas ratio is a key determinant of bacterial growth under MAP and most studies had limited control of this parameter. Devlieghere and others (1998) derived an equation from the gas laws to determine the amount of CO_2 dissolved into product as a function of the product:gas ratio and the amount of CO_2 in a gas mix:

Equation 1: $CO_2^E = ((G/P.\rho + K) - Sqrt((G/P.\ \rho + K)^2 - (4.K.G/P.\%CO_2.\ \rho/100)))/2$

Where CO_2^E = CO_2 dissolved in the product at equilibrium (cm^3/g), G/P = gas:product ratio, ρ = density of CO_2 (g/L), K = Henry's constant (g/Kg atm).

We have reported results of a trial on the effect of some gas mixes on the spoilage of fillets of king (Chinook) salmon (*Oncorhynchus tshawytscha*) (Fletcher and others 2002). We have also demonstrated that increasing levels of CO_2 resulted in decreasing levels of bacterial growth but that high levels of CO_2 gave a "carbonated" flavor (elsewhere described as sherbet, or spritzig) (Fletcher and others 2003). We now describe an experiment to establish the optimum levels of CO_2 for the storage of king salmon and test the applicability of Equation 1.

Materials and Methods

Salmon were starved for 3 d, commercially harvested using the anesthetic AQUI-S™, bled by cutting the gill, rapidly chilled in ice slurry and kept in ice until packaging. They were filleted the next day, and portioned and packaged 2 d after harvesting. Portions were cut from defined positions of the fillet, placed on a drip pad and trimmed to fill a 3.8 x 3.8 x 1.9 cm (i.d.) potting box. This produced a standardized nominal flesh size of 27.4 cm^3 and an exposed surface area of 14.4 cm^2. The box was placed in a foil bag (H030, Cryovac New Zealand Ltd, Hamilton, New Zealand: laminated metallized polyester/EVOH, 82 μm, OTR = <0.1cm^3/m^2/atm/24 h at 23°C, 75% RH, MVTR = <1g/m^2/24 h at 38°C, 90% RH) with an oxygen scrubber, vacuum packed and controlled volumes (0-100 cm^3) of CO_2 or 100 cm^3 of a 40:60 mixture of CO_2:N_2 were hand-injected through a septa and the bags resealed, ensuring minimal leakage during storage. Bags were stored in melting ice (0±0.05°C) and sampled after 8, 22 or 90 d. On each occasion raw sensory characteristics, ATP catabolites, TVBN, drip loss, E_H, pH, instrumental textural measurements, sulfide producer and total aerobic plate counts (APC) were determined on portions in 4 bags from 4 fish from each treatment as described previously (Fletcher and others 2002). A 10-person trained sensory evaluation panel evaluated the cooked flesh using the form described previously (Fletcher and others 2002) and also scored whether the salmon was spoiled (2), slightly spoiled (1) or not spoiled (0). Oxygen and CO_2 levels in the headspace were determined. Colonies counted in the APC were separated by colony morphology and cultures identified by phenotypic characteristics (Holt and others 1994). An experimental set-up similar to that of (Zhao and Wells 1995) was used to determine Henry's constant (K) on samples from 6 fish. A digital pressure meter (Druck Inc. Connecticut, USA; DPI 705) was attached directly to the lid of a stainless steel chamber (volume 408.93 cm^3). A weighed tissue sample was placed in the chamber and left chilled (0°C). After 2 h, the chamber was evacuated (≤ 32 hPa absolute) and flushed with 100% CO_2 3 times to a final pressure of 1100-1200 hPa). The change in pressure was recorded over 96 h by which time equilibrium had been reached. Tissue volume (and density) were determined using a gas pycnometer (Stec Inc., Japan, Model VM-100-E1). K (mole/Kg atm, 0°C) was calculated as follows:

Equation 2: $K = [(P_1 - P_2)(V_1 - V_2)]/ P_2TRS$

where P_1 = initial pressure (atm), P_2 = equilibrium pressure (atm), V_1 = chamber volume (L), V_2 = sample volume (L), T = temperature (°K), R = Reynolds constant (0.082057 L atm/mole °K).

Results and Discussion

Carbonated flavor showed a linear response to increasing levels of 100% CO_2 on days 8 and 22 (Figure 1b) and the data was combined to fit a straight line:

Equation 3: $CF = 0.4914(G:F) + 0.0830$

where CF = mean carbonated flavor score and G:F = gas:fish ratio (cm^3/g).

At 90 days the response was smaller and non-linear (Figure 1a) as other off-flavors masked the carbonated flavor. Panelists gave fresh salmon a mean carbonated score of 0.43 so CO_2 levels giving less than this (a CO_2:Fish ratio of 0.70 cm^3/g) should be acceptable with respect to carbonated flavor. The mean Henry's law constant (K) for CO_2 in salmon tissue at 0°C was calculated as 3.115 g/kg atm (S.E. = 0.0474). Substituting into Equation 1 gave the amount of CO_2 dissolving into the flesh in the CO_2:N_2 mix as 8.61 mL/portion or a CO_2:Fish ratio of 0.310 cm^3/g. Using this value to plot the combined day 8 and 22 carbonated flavor scores for the CO_2:N_2 mix was above the line but not significantly different ($P>0.05$) from the result predicted using 100% CO_2 (Figure 1b). Other sensory scores showed minimal effects with increasing CO_2 except for aggregate scores such as overall spoilage which were probably influenced by the carbonated flavor. Bacterial counts decreased with increasing CO_2. The microbial flora at 90 d was dominated by *Serratia liquefaciens* in vacuum packs, but this decreased with increasing CO_2, being replaced by increasing numbers of *Lactobacilli* (100% with 80 or 100 mL added CO_2). Drip loss increased in the range of 20-40 mL added CO2 and this is attributed to the strong vacuum that was pulled on these bags as all the CO_2 absorbed into the flesh. Instrumental texture analysis demonstrated some softening of the flesh with increasing CO_2. E_H became more negative with time and hypoxanthine increased but neither showed consistent trends with increasing CO_2.

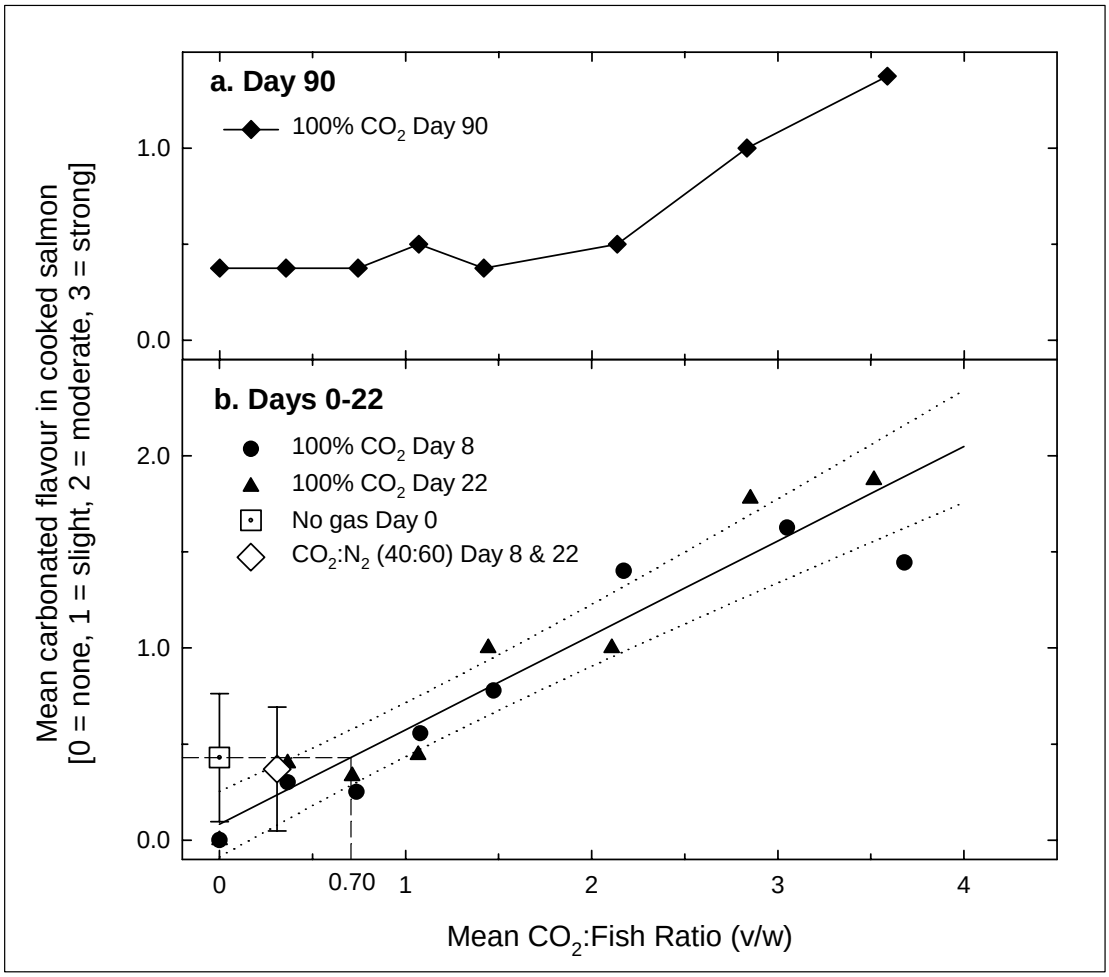

Figure 1 - Effect of different levels of CO_2 on the carbonated flavor observed in cooked salmon. Line fitted (with 95% confidence intervals) to all Day 8 and Day 22 data from 100% CO_2. CO_2:Fish ratio for CO_2:N_2 mix calculated from the formula of Devlieghere and others (1998). Error bars depict 95% confidence limits.

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L 12. EFFECT OF PRE-RIGOR FILLETING AND OTHER PROCESS PARAMETERS ON SMOKED SALMON QUALITY

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Abstract

The objective of this study was to investigate the effect of pre-rigor filleting and other process parameters on the quality of smoked salmon. A total of 208 salmon slaughtered on the same day were purchased from a commercial farm in Norway. Half of the fish was filleted pre-rigor on the day of harvest, while the other half was filleted post-rigor after 4 days on ice. The fish was processed using fully controlled experimental facilities for salting and smoking of salmon. After filleting and trimming of the salmon, different processing parameters were investigated i.e. two different salting methods (injection and dry salting), two different salt contents and two different drying/smoking temperatures. The fillets were measured through the whole process with regard to process yield, gaping, and colour. Furthermore, a shelf life study was carried out on the smoked salmon. In this study vacuum packed samples of smoked salmon were stored for 1, 2, 4, and 6 weeks at 4°C. During the shelf life study the smoked salmon fillets were measured with regard to colour, textural properties, microbiology, sensorial analysis, liquid loss, pH, microstructure, salt, phenol, and fat content. The results showed that the gaping score in both fresh and smoked fillets was significantly higher in post-rigor fillets than in the pre-rigor fillets. Injection salting also resulted in significantly higher gaping score in both salted and smoked fillets. Pre- and post-rigor filleting was found to have a significant effect on the colour of the fresh fillet. However, the effect on colour disappeared during processing. Injection salting had a significant effect on the colour of fillets; this was true both during processing and storage of the fillets. Pre- and post-rigor filleting did not have a great effect on the textural properties of the fresh fillets, while other process parameters were observed to have some effect on the textural properties of the smoked salmon.

Introduction

Traditionally Atlantic salmon (*Salmon salar*) is processed after rigor mortis has resolved, usually 3 to 5 days after postmortem storage on ice. This has been done to avoid handling the fish in rigor and to facilitate the removal of pin bones. Recent studies show that pre-rigor filleting improves several quality parameters in fresh salmon products (Skjervold *et al.* 2001). Furthermore, Einen *et al.* 2002 have studied the effects of freezing pre-rigor fillets on important quality parameters such as texture, fillet gaping, colour, drip loss and yield. However, the effect of pre-rigor filleting on the quality of the product after salting and smoking has not been previously studied.

The objective of this study was to investigate the effect of pre-rigor filleting and other process parameters on the quality of smoked salmon. The yield and important quality parameters such as; gaping, colour, texture and sensory characteristics were studied.

Materials and Methods

A total of 208 salmon slaughtered on the same day were purchased from a commercial farm in Norway. Half of the fish was filleted pre-rigor on the day of harvest, while the other half was filleted post-rigor after 4 days on ice. The filleting was performed using manual filleting. The pre-rigor filleting was carried out in Norway, while the post-rigor filleting was performed in France. The fish was processed using fully controlled experimental facilities for salting and smoking of salmon. After filleting and trimming of the salmon different processing parameters were investigated i.e. two different salting methods (injection and dry salting), two different salt contents and two different drying/smoking temperatures. The fillets were measured through the whole process with regard to process yield, gaping, colour, texture and microstructure. Furthermore, a shelf life study was carried out on the smoked salmon. In this study vacuum packed samples of smoked salmon were stored for 1, 2, 4, and 6 weeks at 4°C.

Fillet gaping in fish is ascribed to the failure of the connective tissue to hold the muscle fibres together. Gaping was measured visually as the length of slits in the anterior-posterior direction of the fillet. Gaping was measured using a scale from 1 to 4, on both the dorsal and ventral fillets, where a score of 1 represents no gaping, 2 minor gaping (gaping occurred over an area covering a length of 1-5 cm of the fillet), 3 moderate gaping (6-10 cm) and 4 much gaping (>10 cm).

Colour was measured using Hunterlab, which uses a tristimulus L*a*b* measurement mode that relates to the human response to colour, were the L* variable represents lightness (L* = 0 for black, L* = 100 for white), the a* scale represents the intensity in red and the b* scale represents the intensity in yellow score.

The **expressible liquid** content was measured, as the quantity of liquid squeezed from smoked samples upon compression. This measurement gives an indication on the water holding properties of the muscle. Measurements of expressible moisture were performed using a Texture Analyser TA.XT2® (Stable Micro Systems, Haslemere, Surrey, UK), by a flat ended probe, 100 mm in diameter, at 50% deformation.

The **textural measurements** were based on the application of the TA.XT2® Texture Analyser and a Warner-Bratzler shear blade was applied. The maximum peak force (N) required to shear through the sample was recorded as shear force.

The samples for the study of the **microstructure** were taken from the white muscle area of the salmon. The method described by Sigurgisladottir *et al.* (2001) was used for the evaluation of microstructure.

Sensory evaluation: A descriptive test with conventional profiling was carried out to evaluate the sensory characteristics of smoked salmon. Samples were scored by twenty two panellists that had been trained on sensory descriptors for smoked salmon. The descriptors used related to the appearance, odour, flavour and texture of smoked salmon slices. An experimental design was built in order to balance various parameters (pre- or post-rigor filleting, kind of salting and level of salt content, smoking temperature). Four smoked salmon samples were presented in each session. Panellists rated the sensory attributes on a continuous unstructured line scale from low intensity (0) to high intensity (10). Products were assigned 3-digit numbers, randomised and served simultaneously

Results

The results showed that filleting the fish pre-rigor had a significant effect on the raw fillet morphology, the pre-rigor fillets were shorter, wider and thicker. Furthermore, a difference in fillet weight was observed, but this can probably been explained by the difference in trimming between the two teams in Norway and France. Results on the microstructure of the fish muscle indicate the muscle cells in the pre-rigor filleted salmon have a larger cross-sectional area. These results are consistent with the dimensions of the fillets i.e. cells are shorter and wider in a contracted muscle.

Pre-rigor filleting had some effect on yield during processing, especially in the salting step. Filleting pre- or post-rigor had a significant effect on the colour measured with Hunterlab. The yellowness score (b value) was significantly higher for the pre-rigor filleted fish. This was true both in the fresh fillets and immediately after salting and smoking. Pre-rigor filleting was also found to have a significant effect on the redness score (a-value) of the fresh fillet, however this effect disappeared during processing and storage. The results also showed that the dorsal gaping score was significantly higher in the post-rigor fillets than in the pre-rigor fillets. This was true both in the fresh fillets, and immediately after salting and smoking. The water holding properties (measured as expressible moisture) of the pre-rigor filleted fresh salmon were inferior compared to the post-rigor filleted fish. After smoking the expressible moisture was significantly higher from pre-rigor filleted fish after one week of storage, however no significant difference was observed between pre- and post-rigor filleted fish during further storage of the smoked salmon.

Pre- and post-rigor filleting did not have a great effect on the toughness/firmness of the fillets. The results from the sensory analysis showed that pre- and post-rigor filleting did not have a large effect on the sensory characteristics of the smoked salmon.

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L 13. BIOPRESERVATION OF COLD-SMOKED SALMON BY THE USE OF SELECTED *CARNOBACTERIUM* STRAINS

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Abstract

The aim of this study is to develop a biopreservation strategy of cold-smoked salmon related to *Listeria monocytogenes* risk, by the use of lactic acid bacteria belonging to the *Carnobacterium* genus and previously selected for their production of antilisterial bacteriocin.
C. divergens V41, *C. piscicola* V1 and *C. piscicola* SF668 were screened for their antagonist effect against a collection of 57 *L. monocytogenes* representative of the strains encountered in the French smoked-salmon industry, using a Petri dish semi-quantitative test. All the Listeria were inhibited but three different groups could be distinguished differing in sensitivity to the three *Carnobacterium*. However, *C. divergens* V41 had always the highest inhibitory effect. The inhibition was then tested in sterile cold-smoked salmon blocks co-inoculated by *Carnobacterium* spp. (10^5 ufc/g) and *L. monocytogenes* (20 ufc/g). *C. divergens* V41 was the most efficient strain, maintaining the level of *L. monocytogenes* inferior to 50 ufc/g during the 4 weeks of vacuum storage at 4° and 8°C, whatever the group of *L. monocytogenes* tested. None of the three *Carnobacterium* showed any spoiling capacity (TVBN production, acidification, off-odour production). Finally, potential application on commercial cold-smoked salmon was tested by spraying *C. divergens* V41 on the slices (10^{4-5} ufc/g) of four different batches freshly processed. When the natural contaminating flora was initially weak (two batches < 20 ufc/g), *C. divergens* V41 quickly reached 10^{7-8} ufc/g and a slight inhibition of endogenous flora was observed. The presence of *C. divergens* V41 was perceived (odour and flavour) but none of the sample was considered as spoiled by the sensory panel. When the natural contamination was initially high (2 batches > 10^{4-5} ufc/g), no effect on the micro-flora nor on the sensory characteristics was observed. In conclusion, *C. divergens* V41 is a good candidate for control of *L. monocytogenes* in cold-smoked salmon.

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L 14. ANTILISTERIAL *CARNOBACTERIUM* SPECIES FROM THE INTESTINAL TRACTS OF ALASKA PINK SALMON: CHARACTERIZATION, BACTERIOCIN PRODUCTION AND COMPETITION AGAINST *LISTERIA INNOCUA*

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Abstract

The objectives were to: (1) isolate and characterize novel, antilisterial *Carnobacterium* species from the intestinal tracts of Alaska salmon, (2) determine bacteriocin activity by two *Carnobacterium* species at 4°C and (3) conduct competitive studies against *L. innocua* in laboratory media and fish extracts. Salmon intestines, collected after fish processing, were diluted and plated on modified MRS agar. Isolated colonies were identified using several identification techniques. Selected isolates were screened for bacteriocin activity using a well diffusion assay against strains of *L. monocytogenes* and *L. innocua*. Competitive studies were conducted by adding 10^3 or 10^6 cells of *C. divergens*/ml and 10^3 cells of *L. innocua*/ml in modified MRS broth (MRS7m) and cooked salmon extracts. Viable bacterial counts were done using modified Oxford agar for *L. innocua* and CTSI agar for *C. divergens*. Antilisterial bacteria were isolated from the intestinal tracts of Alaska pink salmon. Bacteriocin activity was confirmed due to heat-resistance, protease sensitivities and absence of hydrogen peroxide production. Strains having the most bacteriocin activity in culture were confirmed as *C. piscicola* and *C. divergens*. Bacteriocin production was highest in MRS7m broth as compared to standard bacteriological media. Bacteriocin activity in the assay did not require the presence of Tween 80. Two strains produced bacteriocins at 4°C in MRS7m broth and in fish extract containing salt. Maximum specific bacteriocin activity occurred during late log phase to early stationary phase. Both strains inhibited *L. innocua* and bacteriocin was produced in competitive experiments. Bacteriocinogenic *Carnobacterium* species isolated from wild Alaska salmon may be useful against *L. monocytogenes* contamination of ready-to-eat seafood.

Introduction

Listeria monocytogenes is the bacterium of urgent concern due to its ability to infect high-risk, immunocompromised persons resulting in high lethality. Ready-to-eat foods such as soft cheeses, hot dogs, sliced meats and smoked seafoods are susceptible to post-processing contamination and listeria may grow during storage under refrigeration. Bacteriocins have been studied for many genera and species with most of the research conducted on lactic acid bacteria. These low molecular weight peptides can form pores in susceptible bacterial cell walls to result in death. *Carnobacterium* species have been the focus of research activities recently and have been thought to be good candidates for biocontrol of pathogens such as *L. monocytogenes* (Duffes et al., 2000; Nilsson et al., 1999). These probiotic organisms are non-aciduric and can grow at refrigeration temperatures. Ideally, the carnobacteria would be successful in eliminating the pathogens without causing deleterious sensory effects to the food. Some strains have been isolated from meats but current research is investigating fish as sources of carnobacteria (Ringø et al., 2001). The objectives of this project were to isolate and characterize novel, antilisterial *Carnobacterium* species from the intestinal tracts of Alaska salmon, determine bacteriocin activity by two *Carnobacterium* species at 4°C and conduct competitive studies against *L. innocua* in laboratory media and fish extracts.

Materials and Methods

Salmon intestines, collected at commercial fish processors, were serially diluted and spread-plated on de Man-Rogosa-Sharpe agar (MRS; Difco Lab., Detroit, MI) modified to pH 7 (MRS7m; Himelbloom et al., 2001). Isolated colonies were picked, purified on MRS7m agar and identified using several identification techniques including API 50 CHL (bioMérieux Vitek, Inc., Hazelwood, MO) and MicroLog 2 (Biolog, Inc., Hayward, CA) taxonomic systems. Selected isolates were screened for bacteriocin activity using a well diffusion assay (Himelbloom et al., 2001) against strains of *L. monocytogenes* and *L. innocua*. Bacteriocin activity was calculated as arbitrary units per ml (AU/ml) or as specific activity (AU/OD). Growth and bacteriocin preliminary experiments were done using commercial broth media and modified media (pH adjusted and components omitted). Competitive studies were conducted by adding 10^3 or 10^6 cells of *C. divergens* #10/ml and 10^3 cells of *L. innocua* ATCC 33090/ml in MRS7m broth and in cooked salmon extracts stored at 4°C. Aliquots were serially diluted in 0.1% peptone water and 0.1 ml spread-plated on modified Oxford agar (Difco) for *L. innocua* and cresol red thallium acetate sucrose inulin agar (CTSI; Wasney et al., 2001) for *C. divergens*. Colonies were enumerated after 48 hr at 30°C for *L. innocua* and 96 hr at 25°C for *C. divergens*.

Results and Discussion

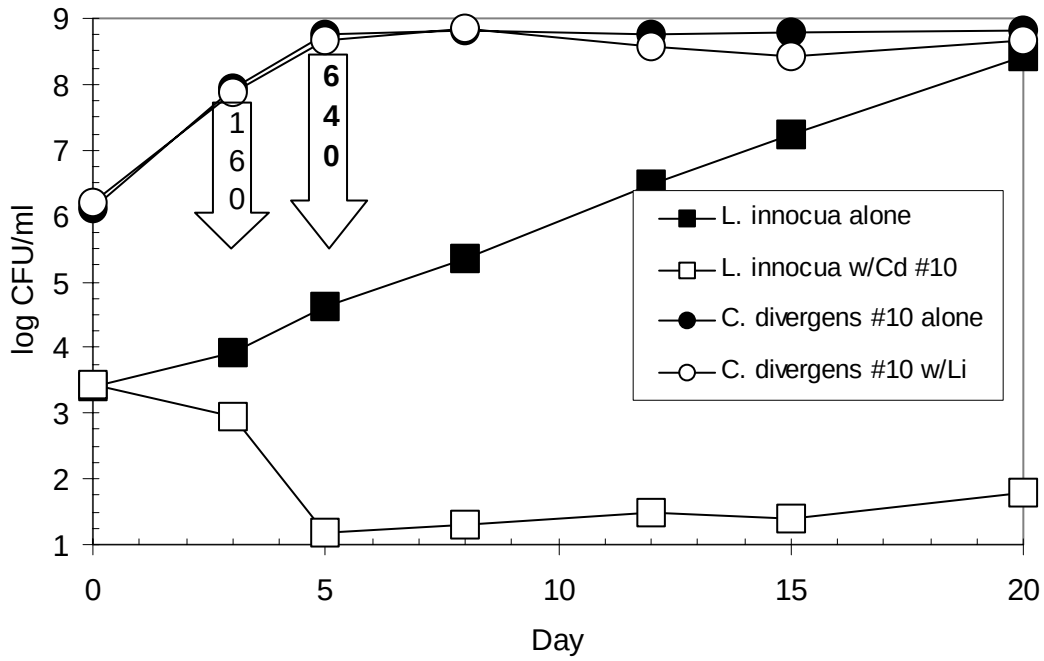
Antilisterial bacteria were isolated from the intestinal tracts of Alaska pink salmon. Strains having the most bacteriocin activity in culture were confirmed as *C. divergens* and *C. piscicola*. Bacteriocin activity was confirmed due to heat-resistance, protease sensitivities and absence of hydrogen peroxide production. Bacteriocin production was highest in MRS7m broth as compared to standard bacteriological media (Table 1).

Table 1. Media Influence on Bacteriocin Production (AU/ml) at 30°C

Media	*C. divergens* #10		*C. piscicola* #7	
	24h	48h	24h	48h
MRS7m	160	80	320	640
MRS9	40	40	0	40
TSB	0	0	40	40
Elliker	20	0	20	20
APT	0	0	0	0
BHI	0	0	0	0

Bacteriocin activity in the well-diffusion assay did not require the presence of Tween 80. Two strains, *C. divergens* #10 and #12, produced bacteriocins under refrigeration in MRS7m broth and in fish extracts containing 0-4% salt. Maximum specific bacteriocin activity occurred during late log phase to early stationary phase and was highest in MRS7m minus glucose (~1400 AU/OD). Both strains inhibited *L. monocytogenes*, *L. innocua* and other strains of carnobacteria. Bacteriocin was produced at 4°C in competitive experiments in cooked salmon extract with salt (Figure 1).

Figure 1. Competition Between *C. divergens* #10 and *L. innocua* in Cooked Salmon Extract Containing 2% Salt at 4°C (values in AU/ml)



Conclusions

Bacteriocinogenic *Carnobacterium* species isolated from wild Alaska salmon may be useful against *L. monocytogenes* contamination of ready-to-eat seafood. Future experiments will consist of cold-smoked salmon inoculated with the pathogen surrogate *L. innocua* and the probiotic bacterium *C. divergens* or *C. piscicola*.

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L 15. EFFECT OF ACTIVE PACKAGING ON SEABREAM (*SPARUS AURATA*) QUALITY

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Abstract

Active packaging is a novel concept of packaging technology, which could extend the shelf life and improve safety during the distribution chain and facilitate the consumption of foods. Therefore, the application of this technology could provide a great diversity of food products and meets consumer requirements for safe, healthy and convenient food. One technique of active packaging includes gas scavengers or emitters, such as oxygen absorbers sachets. There are not many applications of this technique, particular in fish products. In order to assess its effectiveness on fish products, the chilled storage of gutted seabream packed in air including O_2 absorbers, was studied. The quality was evaluated by sensory, microbial and chemical methods. In general, the results pointed out some positive effects of O_2 absorbers, mainly on the appearance and lipid oxidation delay, although some chemical and microbiological aspects need to be well understand.

Introduction

Consumer demand for fresh fish is increasing the significance of gilthead seabream (*Sparus aurata*) as one of the most important fish species farmed in the Mediterranean countries. Farming of this species in Portugal grew in the last years, mostly marketed fresh and ungutted. The seasonal growth of this species in the summer months generates an abundant supply of fresh seabream that has to be commercialised in a short time. On the other hand, there is a clear consumer demand for fresh convenient products that offer healthy, tasty and fast meal solutions. Therefore there is a need to look for new ways of marketing gilthead seabream. Active packaging could extend the shelf life, improve safety during the distribution chain and facilitate the consumption of foods. Techniques include mechanisms to delay the deterioration inside the package, such as oxygen absorbers. The aim of the present study was to evaluate the effect of this technology on the quality of scaled and gutted farmed seabream during chilled storage.

Material and Methods

Raw Material and Experiments

Immature gilthead seabream raised in salt ponds (Timar, Tavira) and fasted for 48 h, was slaughtered by immersion in an ice and sea-water slurry. After death the fish was kept in ice until arrival at IPIMAR. At laboratory the fish was scaled, gutted and washed. The average weight of the individuals was 222.6 ± 20.8 g. Two seabreams (nearly 445g) were placed inside a polypropylene tray, which was put inside a gas barrier bags (transmission rates of 25.0 for O_2; 61.0 for CO_2 and 8.8 for N_2, cc/m^2/24h, at 75% RH and 23°C). In one set of packages O_2 absorbers sachets (O-Buster, type FT) was included (300 cc per package) - batch FTE. The others constitute the control batch - CAE. All packages (v = 2 635 cc) were immediately sealed on a Multivac A 300/52 machine and stored at 4.9 ±0.3ºC.

Methods

At each sampling day two packages of each batch were taken. The gas composition inside the packages was measured with a gas analyser ABISSPRINT. Sensory assessment was done in raw seabreams by a Quality Index Method (QIM) scheme, developed for this species. Microbial counts were done by incorporation in Plate Count Agar and incubation at 30°C for 72 h, under aerobic and anaerobic conditions. A mince was prepared for pH value and chemical determinations. The pH was measured directly on mince, using a surface electrode. Total volatile bases nitrogen (TVB-N) and biogenic amines were determined in 10% trichloracetic acid extracts, according to Cobb *et al.* (1973) and Veciana-Nogues *et al*. (1995), respectively. Nucleotides and nucleotide catabolites were extracted with 0.6M perchloric acid (Mendes *et al*., 2001) and quantified according to Ryder (1985). K-value was calculated according to Saito *et al*. (1959), using inosine monophosphate, inosine and hypoxanthine concentrations. Peroxide value (POV) and malondialdehyde (MDA) were determined according to AOAC (1990) and the method described by Vyncke (1970/72), respectively. All analyses were carried out at least in duplicate.

Results and Discussion

The main changes in gas composition (data not shown) occurred in batch containing O_2 absorbers (FTE), within the first two days of storage, when O_2 decreased to a concentration of 8.6% (±0.8), corresponding approximately to a 54% of oxygen absorption inside the package. At 6th day, O_2 levels attained 0.0% (±0.1) and remains almost constant until the end of storage. In the CAE batch, O_2 decreased very slowly from 19.4% (± 0.3) to 13.6% (± 0.1) after 6 days. The changes on CO_2 were not so noticeable, the highest concentration was observed for CAE lot, which values raised up to 12.7% (± 0.1) at 10th day, mainly due to a higher microbial activity. Effectively, after the 3rd day microbial counts (data not shown) were higher in CAE batch than in FTE, respectively $1.8x10^8$ and $4.2x10^5$ cfu/g, at day 10. However, counts from anaerobic incubation were quite similar, $2.6x10^5$ cfu/g for CAE and $1.6x10^5$ cfu/g for FTE lot.

The K value (Fig. 1) increased from 12% at initial to 45% and 50%, at the end of storage period, respectively for FTE and CAE lots. The linearity was higher in the batch without O_2 absorbers ($K=3.98t+14.7$, $R^2=0.9603$; where K is K value and t time of chilled storage) than in that with absorbers ($K=3.34t+16.9$, $R^2=0.808$). K value changed faster and attains higher values than published data for the same species (Huidobro *et al*., 2001.)

The pH (data not shown) was initially 6.25 and changed during chilled storage to 6.30 (±0.1) and 6.43 (±0.2), respectively in lots FTE and CAE.

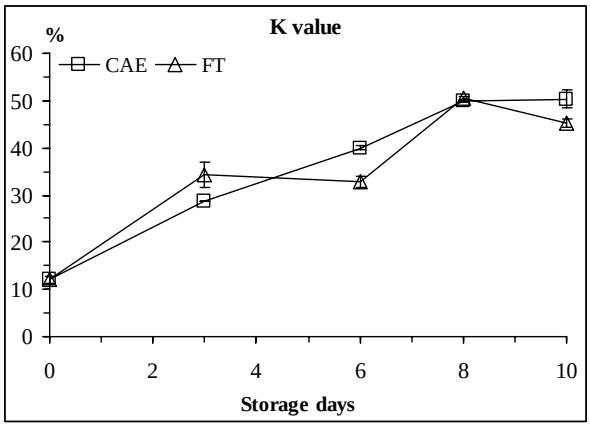

Figure 1. K value of seabream packed in air, stored at 4.9 ±0.3ºC. CAE (control), FTE (containing O_2 absorbers). Error bars are the standard deviation (n=2).

TVB-N (data not shown) levels close to 19 mgN/100g (±0.9) were found at the beginning. During chilled storage a small but steadily increase was observed in both batches, which attained contents around 25 mgN/100g (±0.0) after 10 days. Connell (1995) refers a range between 5 and 20 mg TVB-N/100g in freshly caught fish, and levels of 30-35 mg/100 g as the limit of acceptability for iced stored cold-water fish. Production of biogenic amines was not observed in both lots.

In what concerns to lipid oxidation, a regular increase both in POV and MDA contents took place during the storage period, but lower values were registered in the lot containing O_2 absorbers. The highest POV content was attained at day 8, 52±4.9 and 38±1.5 Meq/kg, respectively for CAE and FTE batches. Initial value of MDA (Fig. 2) was around 0.5 mg/kg and at the end of the storage the level of 8 mg/kg, at which rancid flavours became evident in fish (Connell, 1995), was not reached in both lots.

Regarding to sensory evaluation, significant differences between the two batches were not observed (p=0.614), although the FTE lot registered lower demerit points at the end of storage (Fig. 3), mainly due to the better appearance.

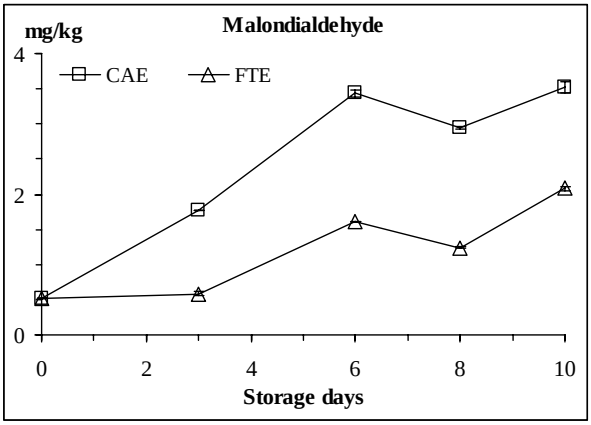

Figure 2. Malondialdehyde content of seabream packed in air, stored at 4.9±0.3ºC. CAE (control), FTE (containing O_2 absorbers). Error bars are the standard deviation (n=2).

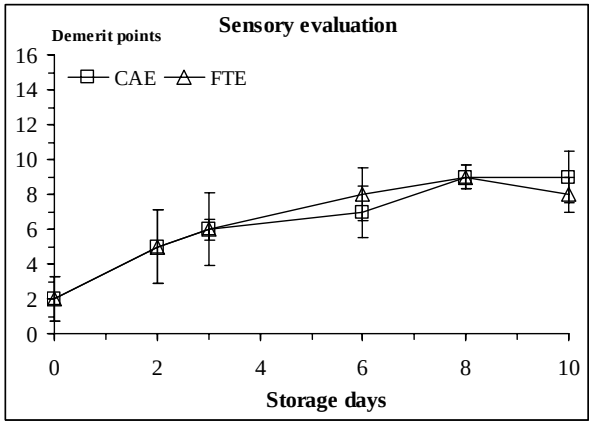

Figure 3. Sensory changes of raw seabream packed in air, stored at 4.9±0.3ºC. CAE (control), FTE (containing O_2 absorbers). Error bars are the standard deviation (n=5).

As a conclusion, it can be said that O_2 absorbers were very efficient in the oxygen reduction. However, results pointed out the need to study in more detail their effectiveness on the preservation of fish quality.

Acknowledgements

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L 16. TECHNICO-ECONOMIC FEASIBILITY OF SOFT-SHELL CLAM (*MYA ARENARIA*) RELAYING, QUEBEC, CANADA.

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Abstract

This project was undertaken to resolve the problem of bacterial contamination and to allow exploitation of soft-shell clam on the Gaspé peninsula. In July 2001, soft-shell clams were harvested in the lagoon of Port-Daniel. Clams were immersed in an approved site designed to test the efficiency of four different types of container and two immersion depths. The cleansing lasted 14 days. During Days 0, 1, 2, 3, 4, 8, 14, samples were collected in the different devices for coliform analysis.

Results show that the initial contamination was low – 80,9 fecal coliforms per 100 g of tissue – but sufficient to prohibit harvest. A fast cleansing was observed in all conditions: clams conformed after 12 h, and they were completely sanitized after 72 h. Comparisons revealed an optimal cleansing with net tubes and oyster pots within 72 h. During the long term – 14 days –, net tubes and larger pots had better performances, and the immersion depth was an important factor with a better cleansing close to the surface. Mortalities were low in all containers – ± 3 %. A significant drop in the product quality was observed from Day 8 with a significant weight loss.

The experience confirmed that relaying is a valid approach to improve rapidly the bacteriological quality of clams. Complementary trials will be necessary to determine ideal conditions for relaying. But it seems already certain that the ultimate choice of a container and a depth will depend closely on the chosen relaying duration. The low mortality rate is a positive result, but the drop in quality and yield produces questions about the short duration relaying. However, the validation of a short duration protocol hinders the governmental obligation to prove its efficiency on strongly contaminated organisms – 20 shellfish lots ≤ 230 F.C./ 100 g – non-existent on the Gaspé peninsula.

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P 1. HIGH-PRESSURE PROCESSING OF COLD-SMOKED SALMON

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Objective:

Preliminary studies of the effects of high-pressure processing on cold-smoked salmon and its effects on quality attributes such as texture, colour and sensory.

Methodology:

Vacuum packed cold-smoked salmon was subjected to high-pressure with varying pressure levels (100-300 MPa), process time (10–30 min) and process temperature (20 – 30 °C) and stored both at refrigerated and room temperature. Quality of the product was evaluated in terms of changes in sensory characteristics, colour, TBA-RS and texture at pre-determined intervals during storage period.

Results:

The shelf life of the salmon was evaluated based on changes in sensory, chemical (TBA-RS), cutting strength and colour parameters during storage period. The present experimental design yielded optimum process variables (pressure level, process temperature and time) for further experiments. At 300 MPa, the cutting strength of the product was increased irrespective of the further storage temperatures. In addition, high-pressure caused an increase in L* values (opaqueness) and a declined red colour intensity (as measured by a* values) was observed with increase in pressure, but still the instrumental values were within the acceptable levels. These changes were very well correlated with visual changes. Lipid oxidation as measured by TBA-RS was also increased as increase in pressure and holding time.

Conclusions:

High pressure at higher pressure levels had caused products with unacceptable quality and the present experiment gave us a way to proceed for subsequent experiments with suitable process parameters.

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P 2. THE EFFECTS OF BRINE INJECTION ON YIELD, WATER HOLDING CAPACITY AND CHEMICAL CONTENT OF COD FILLETS

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Abstract

This summary includes results from two trials (II and III), where cod fillets were injected with brine and then immersed in brine with the same composition for 20 minutes. The composition of the injection brine varied with groups, containing phosphates (3% w/w of brine), salt (5% w/w of brine) and/or proteins (10% w/w of brine). In trial II soya proteins were used but in trial III dried, hydrolysated cod proteins were used. Two groups were used as control groups, in the first one fillets were frozen without treatment and in the second only water was used for brine injection and immersion. The fillets were stored at –24°C for 3 months and then thawed to evaluate the effects of the different brines on yield, drip, water holding capacity, pH and chemical content. The aim was to increase yield and water holding capacity of the fillets, resulting in better quality, such as less dryness after frozen storage.

Addition of proteins slightly increased the yield of thawed fillets but salt and phosphate were more effective. The highest yield in II was obtained when soya proteins, salt and phosphates were used together but in III, when salt and phosphate were used together. These two groups were highest in water content and lowest in protein content. Fillets in the control groups were lowest in yield and contained the highest protein content and lowest water content. Comparison of the different proteins showed that fish proteins were not as effective as the soya proteins. The use of both types resulted in negative appearance of the fillets.

Introduction

Light salting of frozen cod fillets is a process that has been growing in Iceland due to higher demand for such products in Southern Europe. The fillets are light salted by brine injection and /or immersion into brine. The purpose is to enhance stability during frozen storage and increase water holding capacity. After brining the fillets are frozen and exported (Thorarinsdottir and others, 2002).

Functional properties and chemical content of cod fillets are altered by process parameters, like freezing (Shenouda, 1980; Mackie 1993). Seasonal variations in the raw material also affect the chemical content and the functional properties of the muscle (Ingólfsdóttir and others, 1998). Therefore it is an interesting question if added fish proteins from by-products like cut-offs, can be injected to even out seasonal variations in chemical content and functional properties of the muscle like water holding capacity and improve stability during processing.

The objectives of this research were to test the effects of salt, phosphate and added proteins, used separately or together, on factors like yield, drip, water holding capacity, chemical content and pH. The aim was to increase yield and water holding capacity of the fillets, resulting in better quality, such as less dryness after frozen storage.

Materials and Methods

Two trials, II and III were performed, in both trials phosphate (F) and salt (S) were used. Different proteins (P) were used, soya proteins in trial II and dried hydrolysed cod proteins in trial III. The cod (Gadus morhua) was caught by line near the south west coast (Reykjanes) in May 2001 in trial II but near the west coast (Snæfellsnes) of Iceland in May 2003 in trial III.

The fillets were injected with brine at 1 bar pressure (Dorit INJECT-O-MAT, PSM-42F-30I, Auburn NSW, Australia) and then immersed in brine for 20 minutes. The composition of the brine varied, when salt was used it was in 5% concentration, phosphates in 3% concentration and proteins in 10% concentration. The fish to brine ratio was 1:1 and the temperature of the brine 3 ± 1 °C. One group (control) was not injected or brine salted before freezing. The fillets were frozen in a plate freezer (Amerio, Union City, N.J. USA) for approximately 2 hours and then stored at –24 °C for 3 months. Thawing was carried out at 20 °C for 7-8 hours. Parameters evaluated were yield, water holding capacity, chemical content and pH.

Results and Discussion

The yield was increased ($p<0,05$) by the use of phosphate, salt and soya proteins, the use of phosphate had the strongest effects in both trials. However, the fish proteins did not have significant ($p>0,05$) effects, although the results indicated that the yield was slightly increased by adding them to the fillets. The cooking yield was increased by the use of phosphate and salt in trial III but in trial II the values were slightly higher when phosphate was used with or without salt or soya proteins.

The strongest effects on water holding capacity were because of increased salt concentration in the muscle but phosphates did also have significant ($p<0,05$) effects in trial II. The salt content in fillets injected with brine

containing salt, was in the range of 0.8-1.1%. Fillets in other groups contained 0.1-0.3% salt, which was similar as in the control group which was not treated before freezing.

The protein content was negatively affected by the use of salt and phosphate in both trials but significantly increased by the use of fish proteins in trial III. Adding proteins to salt and/or phosphate brines led to lower water content in the injected fillets. It is known that water and protein content are negatively correlated as could been seen in these trials. As the amount of water in the muscle increased, the ratio of dry material, including proteins decreased and vice versa. However, the content of dry material may have been affected by loss of proteins and water-soluble compounds during brining (Callow, 1931; Zaitsev and others, 1969.

It should be pointed out that some variation was noticed in the condition of the raw material between the trials, which may have affected the results. It should be kept in mind comparing values for fillets treated with soya and fish proteins, but groups treated the same way in both trials did also give indications about differences caused by variations in the raw material.

Conclusions

The effects of soya and fish proteins on yield were similar when only proteins were added to the fillets, with injection and brining. However, the results indicated that the fish proteins had less effects when used with salt and/or phosphate. The appearance of the fillets was negatively affected by both types of proteins.

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P 3. THE EFFECT OF FRESHNESS AND STORAGE METHOD ON SALTING OF SALMON (*SALMO SALAR* L.)

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Abstract

In order to meet the increasing demand for high quality fish products, the physicochemical properties of salmon (*Salmo salar*, L) during storage and salting were investigated. Textural properties, denaturation of fish proteins (as changes in protein solubility and molecular weight by SDS-PAGE) as well as water holding capacity of salmon fillets were measured after 2, 8 and 15 days period of chilled, super-chilled and frozen storage followed by two weeks of dry-salting.

Salting was found to cause an increase in the hardness of fillets in all of the different raw materials. In general, the amount of extractable proteins were reduced during storage and salting up to 14 days in those fillets that had been stored frozen or chilled, mostly due to the decreasing amount of the salt soluble proteins. However, light salted salmon had low variation in the total amount of extractable protein compared to heavy salted salmon. On the other hand, the super-chilled storage salmon showed higher solubility of the proteins and lower variation through the salting process.

In conclusion, there were no large differences on the effect of different freshness of salmon and the type of storage carried out. Nevertheless, super-chilling seems to be a promising preservation method before salting.

Introduction

Over the last years the trend in food industry has moved rapidly from frozen to fresher products. This fact has lead new to challenges for food producers and refrigeration industry since the time and the storage temperature are the main factors to keep the fish shelf life (Torstveit 2002 http://www.fao.org 23/10/02). Traditionally the way of storing and sale-distribution fresh fish, is either in crushed ice or under frozen storage. The main problem of iced storage is that post-mortem auto-degradation of fish muscle cannot be completely stopped which reduces freshness (Kato and others 1974). Furthermore, the transportation of chilled fish results in excessive melting and the required amount of ice also increases the transport cost. Frozen storage is a good alternative to extend the fish shelf-life, however, it causes insolubilization of the myofibrillar protein and loss of functional properties (Mackie 1993). Super-chilling has been industrially used with a few fish species such as tuna, salmon, mackerel, in order to extend freshness-preservation over a few weeks (Alemán 1982). Products are partly frozen (-1^oC to -3^oC) compared to $0/1^oC$ in the chilled storage and super-chilled transport would avoid the melting ice and the extra-weight along the distribution chain.

Atlantic salmon (*Salmo salar* L.) is one of the most important Norwegian fish supplies commercially (Elvevoll and others 1996). Salmon stored in the different ways mentioned above can be used for direct consumption or to porduce processed fish products such us marinated and smoked salmon. Salting of salmon is the first step in the manufacturing chain of the smoking industry.

The purpose of the present study was to compare the influence of the aging of salmon combined with the storage method prior to processing on the quality of the final salted product. Quality changes in texture, denaturation of fish proteins (as changes in the protein solubility and SDS-PAGE) were investigated.

Materials and Methods

Fish Material

Salmar Processing AS supplied farmed gutted salmon with an average weight of (3,35 ± 0,3) kg. After rigor mortis was accomplished for 2 days, it was kept under (iced storage at 4^oC during 0 days (raw material **A**), 7 days (r.m. **B**) and 14 days (r.m. **C**), super chilled storage at -1^oC during 7 days (r.m. **S**) and 14 days (r.m. **R**), frozen storage at -40^oC during 30 days (r.m. **F**)). After the storage treatment, each salmon was filleted, tagged and dry salted with ordinary refined salt, in plastic containers without drainage at 4^oC. Samples for physico-chemical analysis were taken from two different fillets randomly and each fillet was analysed twice at each step. *Light salted salmon* was considered between 12 and 24 hours of salting as the raw material for the smoking industry. *Heavy salted salmon* (14 days salted) was considered as a reference product to study the behaviour of a fatty salted fish product such as herring.

Physico-chemical determinations

Textural properties were measured with a TA.XT2 Texture Analyser by a modification of the method described by Eien and Thomassen 1998. Texture profile analysis (TPA 40%) was carried out and 6 measurements

were run on each fillet. Water content was determined by oven drying for 48 hours at 105ºC. Water holding capacity (WHC) was determined on minced muscle by low-speed centrifugation as described by Eide and others 1982. Water and salt soluble proteins were determined by a modified method after Licciardello and others 1982. Amount of proteins in the extract was determined with BioRad protein assay (Bradford 1976). SDS-PAGE of the salt soluble protein fraction was carried out on a Pharmacia LKB PhastSystem and performed according to Laemmli 1970.

Results and discussion

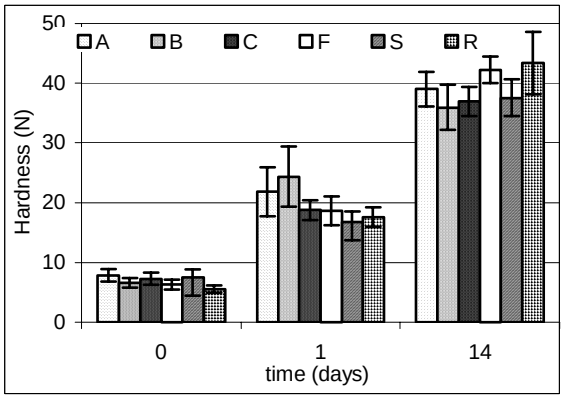

Figure 1. Hardness (N) of salmon fillets after each storage treatment (day 0), after1 day salting and after 14 days salting. A, B, C, F, S, R are the different raw materials use for salting under different storage methods.

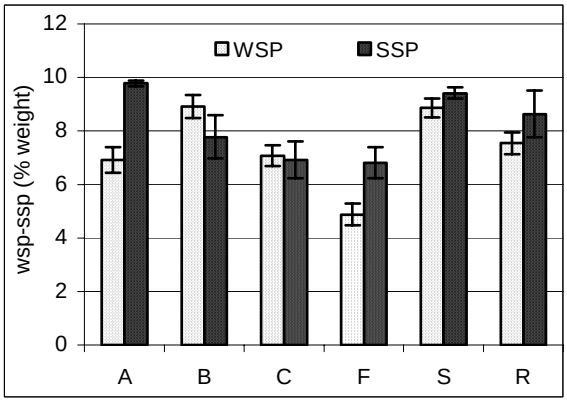

Figure 2. Changes in water soluble (WSP) and salt soluble protein (SSP) depending on the storage methods.

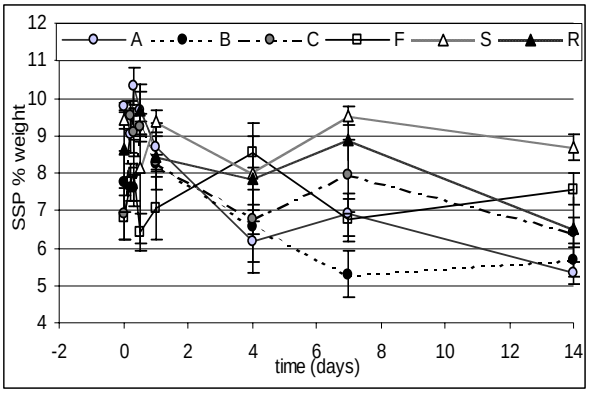

Figure 3. Changes in salt soluble protein (SSP) during 14 days salting depending on the way of storage

Softening of fish muscle during storage was slightly reduced though no significant differences depending on the aging and the storage method were shown (figure 1). Salting cause a gradually increase in hardness of 200% after 1 day of salting and 500% after 14 days of salting. The softer fillets prior salting were the ones treated during frozen storage and two weeks of super chilled storage; nevertheless, after 14 days salting required maximum force.

As shown in figure 2, the amount of extractable proteins was reduced during aging independent of the storage method. It is important to note superchilled treatment (S and R) showed comparable extractability to the chilled storage (A) which is the fresher raw material.

The state of the proteins is dependent on the salt content. Muscle proteins generally first show an increase in solubility (salting-in) with increasing salt concentration. This is followed by a decreased solubility (salting-out) on further addition of salt, mainly due to the protein precipitation (Thorarisndóttir and others 2001, Sigurgisladóttir and others 2000, Hernández-Herrero and others 2000, Hultin and others 1995) as shown in figure 3 the reduction of extractable protein was mainly due to the decrease in salt soluble protein throughout the salting process. Salt that increase the solubility of proteins also tend to denature them. But in the SDS-PAGE there are no marked changes throughout the salting process and the storage method despite the protein precipitation. Lightly salted salmon had low variation in the total amount of extractable protein compared to heavily salted salmon. The super-chilled storage salmon showed higher solubility of the proteins and lower variation along the salting process.

Conclusions

Superchilling seems promising for storing raw material before salting, since biochemical quality degradation that easily happen under chilled storage is slowed down, while the negative effects of frozen storage on proteins was not observed.

Acknowledgements

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P 4. THE EFFECT OF MODIFIED ATMOSPHERE PACKAGING IN EXTENDING QUALITY/SHELF LIFE OF CHILLED FILLET OF EUROPEAN SEA BASS (*DICENTRARCHUS LABRAX)* AND MEAGRE (*ARGYROSOMUS REGIUS)*. PRELIMINARY RESULTS

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Abstract

The aim of this preliminary trial was to examine the effect of MAP application (53% N_2 + 40% CO_2 + 7% O_2) in extending quality/shelf life of European sea bass (Sbmap) and meagre (Mmap) fillet in comparison to fillet from the same fish packaged without map (Sb and M) and to fillet from whole fish of both species (Sbw and Mw). Fillets safety and quality involution at 1°C were evaluated on 63 sea bass (600±86 g b.w.) and 52 meagre (683±170 g b.w.) through: muscular TVC, *Pseudomonas* spp. and lactic acid bacteria, pH and sensorial evaluation (EU scheme) at the 1st, 2nd, 5th, 6th, 7th, 8th day after packaging; malonaldehyde content at the 6th, 7th, 8th day; sensory evaluation of cooked Map and control fillets at the 5th and 7th d after packaging. The lower pH values of Sbmap and Mmap muscle hardly reached the significance. In both species TVC and *Pseudomonas* spp. were significantly lower in Map and whole fish fillets than in control fillets from the 2nd d after packaging. The Sb and M fillets reached values higher than 7-8 log cfu/g already at the 5th day, while Map fillets showed TVC, *Pseudomonas* spp. counts less than 5 log cfu/g at the 8th day after packaging. Malonaldehade did not increased significantly from the 6th to the 8th day and showed lower values in whole fish fillets than in Map ones. Colour of map fillets had generally lower scores than the control fillets while the opposite emerged in odour scores (less spoilage odour). No significant differences between cooked fillets emerged at the 5th day, while low quality but edibility of SBmap and Mmap versus unfit situation of Sb and M fillet emerged on the 7th d.

Introduction

Nowadays it is increasing the awareness of the need in minimising seafood losses, improving food management and preservation and in offering to the market differentiated products and more natural and less heavily preserved food to meet the consumer demand. Modified atmosphere packaging of chilled fish, a technique for extending fresh products quality/shelf life most used in UK and France, seems species dependent, so studies on warm waters species can be useful also because, until now, the technique has found little practical application with fish products in Italy. The aim of the present research was to study quality and quality changes of fillet packed in modified atmosphere in comparison to the correspondent control fillet and to the fillet taken from the whole fish in reared sea bass (*Dicentrarchus labrax*) and meagre (*Argyrosomus regius*), white fish with different fat content in body and muscle.

Materials and Methods

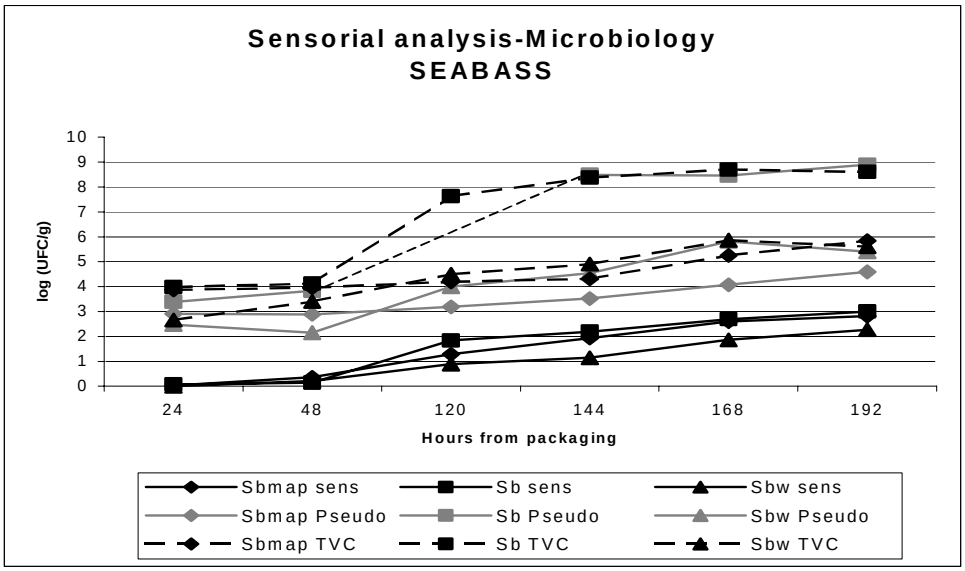
Sixty three sea bass (600±86 g b.w., 268±42 g fillets w.) and 52 meagre (683±170 g b.w., 298±69 g fillets w.) intensively reared at 19-23°C water temperature, 4-7 ppm D.O., 34‰ salinity and fed commercial diet (45% CP, 20% EE, 16% NFE, 19.4 MJ DE/kg) were slaughtered in ice water slurry, transferred in boxes covered by ice to the processing plant by a refrigerated van. The day after death, n. 51 sea bass and 43 meagre were filleted and the right fillets (Sbmap and Mmap) were packed in MAP (53% N_2 + 40% CO_2 + 7% O_2), while the left control fillets were only packed with permeable film covering (Sb and M). The remaining 12 sea bass and 9 meagre were maintained whole (Sbw and Mw). All samples were stored at 1°C (the whole fish covered by ice) until spoilage. For each species were carried out the following determination: sensory evaluation (EU scheme with E class - 0, A class - 1 and B class - 2) and pH measure on 3 MAP and 3 correspondent control fillets and 1-2 whole fish fillets at 1, 2, 5, 6, 7, 8 days after packaging; malonaldehyde content in muscle (Raharjo et al., 1993) on the same fillets sampled at 6, 7, 8 days after packaging; total viable counts at 25°C (TVC), *Pseudomonas* spp. and lactic acids bacteria by MRS at 30°C in anerobic condition on 3 MAP and 3 correspondent control fillets and 1-2 fillets from whole fish; sensorial analysis by triangle test on 3 MAP and 3 correspondent control cooked fillets sampled at 5 and 7 days after packaging. Data were analysed by ANOVA (Species, Storage condition, Time from packaging).

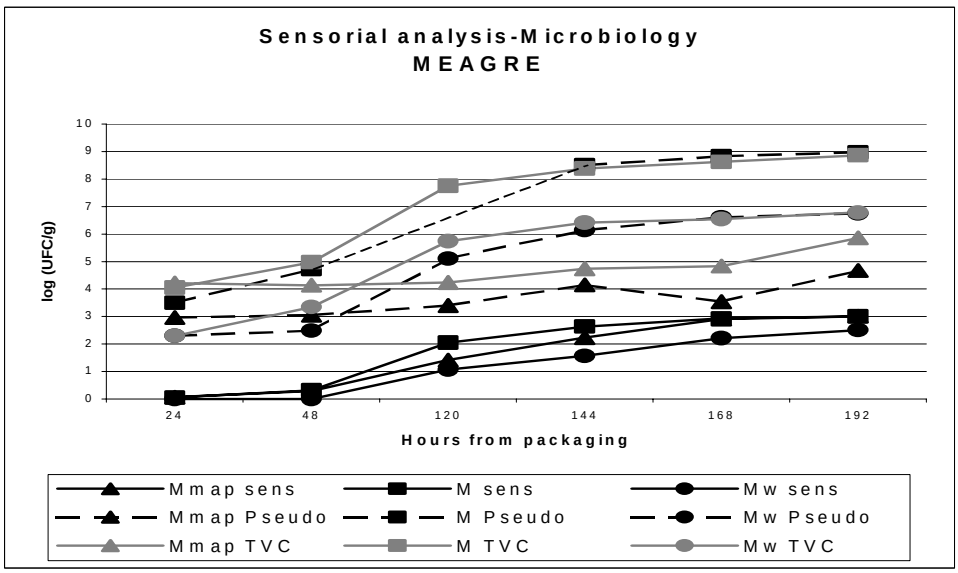
Results and Discussion

Species effect did not emerged while fillet storage condition and time from packaging significantly influenced quality changes of fillet ($P < 0.001$). As showed in figures, at the 1st and 2nd day after packaging SBw and Mw showed the lowest TVC and *Pseudomonas* spp in comparison to the other storage conditions of fillets while at the 2nd day also Sbmap and Mmap had a lower *Pseudomonas* spp. count than the Sb and M fillets; at the

5th, 6th, 7th and 8th day Map fillets showed the lowest values of TVC and *Pseudomonas* spp., while the whole fillets had intermediate and the control fillets the highest levels (> 7- 8 log cfu/g); at the 8th day after packaging Map had TVC and *Pseudomonas* spp. counts less than 5-6 log cfu/g. Lactic acid bacteria counts of Map fillets and whole fish fillets were lower than those in control fillets. Differences between map and whole fish fillet emerged only at the 5th or 6th day after packaging in sea bass and in meagre respectively. Map fillets generally showed lower pH values even if the significance of the difference emerged only at the second day in meagre and at the end of the checking period in sea bass. No significant increase emerged from 6th to 8th day after packaging in malonaldehyde levels, that were lower in whole fish fillets than in map fillets, control fillets showing the intermediate values. Sensorial scores of raw fish indicated a significant best quality of the whole fish fillets and a better quality of Map fillets in comparison to the control fillet at the 5th and 6th day after packaging in meagre and at the 5th and 8th day in sea bass; the odour of fillets indicated the best quality of Sbmap and Mmap fillet at 5th, 6th, 7th and 8th after packaging. Spoilage odour was then well controlled by MAP, thank to the limiting effect on microbial growth. On the contrary, lower aspect and colour values were reported in map fillet in comparison to the whole fish fillets. Panel taste did not found difference between Map and control cooked fillet at the 5th day after packaging, while clear differences emerged at the 7th day when the map fillets were all accepted while the control ones were all rejected. The shelf life extension in comparison to control fillets can be indicated around two days both for sea bass and for meagre even if some individual parameters seemed to indicate better values in sea bass than in meagre.

Results appear in agreement with results on other white fish (Huss, 1995).





Conclusions

The clear differences emerged in microbiological quality between map fillet and the correspondent control fillets from sea bass and meagre were less evident by sensorial analyses, mostly in cooked fish. Little difference emerged between the species in shelf life extension due to the MAP application (1/2 days). Sea bass seemed to maintain the best condition of shelf life and quality parameters.

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P 5. COMBINED EFFECT OF GERMINANTS AND HIGH HYDROSTATIC PRESSURE ON THE INACTIVATION OF *CLOSTRIDIUM BOTULINUM* TYPE E SPORES

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Introduction

High Hydrostatic Pressure (HHP) is a relatively new technology that has the potential to be used as an alternative to thermal processing to extend the shelf-life of foods without compromising their sensory and nutritional characteristics (Tewari *et al*., 1999). Several studies have confirmed the effectiveness of HHP against a wide range of food spoilage and pathogenic microorganisms (Furukawa *et al.,* 2002; Arroyo *et al.,* 1997; Hoover *et al.,* 1989). However, while HHP processing at 300 –700 MPa has had a marked effect on vegetative microbial cells, it has little or no effect on bacterial spores that are capable of surviving pressures >1,200 MPa. This resistance of spores to HHP was attributed to dehydration of the spore protoplast, the presence of spore specific DNA-protecting proteins and barrier properties of the spore coat (Wuytack and Michiels, 2001).

Several methods have been investigated to decrease the resistance of bacterial spores to HHP. These include pressure cycling and the use of heat prior to, or after, high pressure treatment (Mills *et al*., 1998). More recently, Plowman and Peck (2002) showed that various amino acids could be used to enhance the germination of spores of *C. botulinum in vitro.* This approach has the potential to induce germination of spores *C. botulinum* type E, a pathogen of concern in smoked fish products, and enhance the antibotulinal efficacy of HHP.

Objectives

The objective of this study was to (i) optimize the germination of *C. botulinum* type E spores using various germinants and (ii) to observe the combined effect of germinants and HHP on the inactivation of *C. botulinum* type E spores, both *in vitro.*

Materials and Methods

1. Preparation of spore stocks. *C. botulinum* type E spores (strain Bennett) were be used throughout this study. Spores were prepared as previously described (Dufresne *et al.,* 2000).

2. Optimization of Germination Rate. Various concentrations and combinations of spore germinants (L-alanine, L-lactic acid and L-cysteine) were investigated to determine their optimal combination and concentration for maximal germination of *C. botulinum* spores. Spores (~10^8 spores/ml) were incubated with germinants in phosphate buffer, with and without salmon extract, for various combinations of time and temperature. The extent of germination was assessed by phase-contrast microscopy to distinguish phase dark (germinated) and refractile (ungerminated) spores. Rates and extents of germination were calculated and reported as the percentage of spores relative to the total number of spores enumerated.

3. High Hydrostatic Pressure Treatment. The effect of HHP and the optimal germinant solution on heat activated and non-heat activated *C. botulinum* spores was studied. Four combinations were examined: heat-activated and non-heat activated spores, with and without the optimal germinant cocktail. Samples of spores treated with germinants in phosphate buffer were subjected to pressure (200-400 MPa) for various time-pressure combinations in a pre-cooled high-pressure chamber (Autoclave Engineers, Erie, PA, USA). The internal temperature of the chamber was monitored using a thermocouple linked to a Data Acquisition/Switch Unit (Hewlett Packard, Model 34970A). Enumeration of surviving spores was done after each processing/time treatment as described previously.

Results and Discussion

Optimal germination (approximately 95 to 99% of spores) was obtained with a combination of 100 mM L-alanine in combination with 50 mM L-lactate, 50 mM $NaHCO_3$ and 25 mM sodium thioglycollate in 100mM phosphate buffer containing sterile salmon extract. Light and electron microscopy indicated germination of spores but not outgrowth of vegetative cells.

Subsequent studies were done to determine the combined effect of germinants and HHP (200-400MPa) on the destruction of *C. botulinum* type E spores. HHP caused a 2-log destruction of spores that were exposed to germinants without prior heat activation, regardless of the level of pressure (Figure 1). A combination of heat activation and exposure to germinants resulted in greater than 4 log reduction of endospores, again regardless of

pressure. An even higher reduction (up to 4.5 log) occurred when the germinated and pressure treated spores were subjected to a further minimal heat process (60°C for 10 minutes) (Figure 2).

This initial study has shown that HHP has promise as a method to inactivate *C. botulinum* if spores are induced to germinate prior to high pressure treatment.

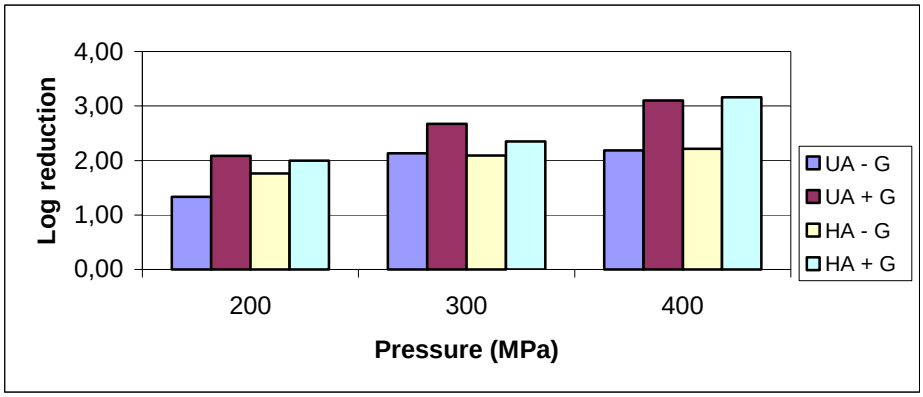

FIGURE 1. Combined effect of germinants and HHP on the inactivation of *C. botulinum type* E spores in salmon extract.
Legend: UA – G, not heat activated, no germinants; UA + G, not heat activated, with geminants; HA – G, heat activated, no germinants; HA + G, heat-activated, with germinants.

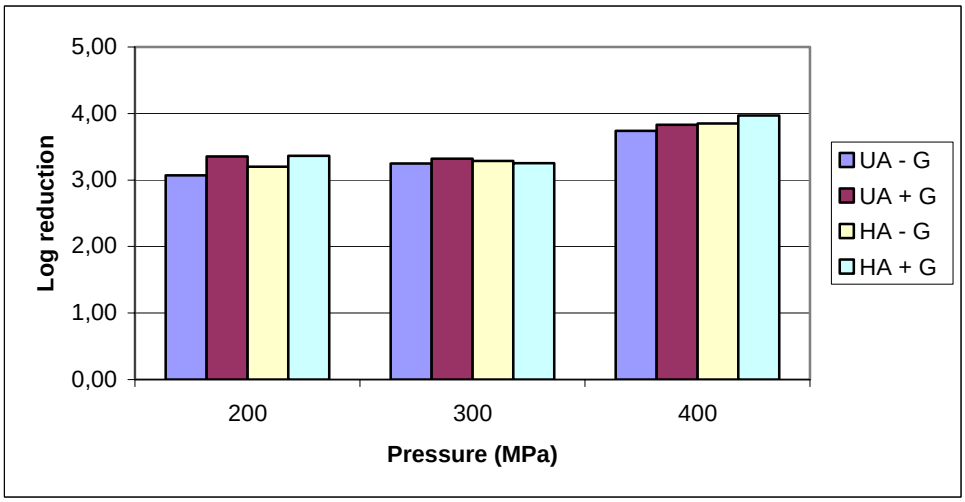

Figure 2. Combined effect of germinants, HHP and heat post-processing on the inactivation of *C. botulinum* type E spores in salmon extract.
Legend: UA – G, not heat activated, no germinants; UA + G, not heat activated, with geminants; HA – G, heat activated, no germinants; HA + G, heat-activated, with germinants.

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P 6. SOUS VIDE/FREEZING OF COD AND SALMON PORTIONS

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Abstract

Tests indicated that sous vide cooking followed by freezing is a suitable technology for cod and salmon portions. Using raw vs previously frozen fish for sous vide cooking had no influence on the test parameters for the cooked products. A long sous vide cook time adversely affected product colour in comparison with a short cook time for both species while freezing post sous vide cooking gave higher flesh shear values for both species in comparison with chilling.

Introduction

The market for ready-meals, including those containing fish, is expanding rapidly in Europe (Sands, 2002) and sous vide cooking is one of a number of options for processing ready-meals. The recommended minimum thermal process for sous vide products is 90^0C for 10 min (Creed & Reeve, 1998). However, concerns about the safety of sous vide chilled products (Rhodehamel, 1992) prompted the current study where freezing was used as an alternative to chilling for sous vide products. Freezing has two advantages: (a) it minimizes the risk of growth of *Clostridium botulinum* spores, and (b) it extends product shelf-life. However, it could lead to increased in-pack drip and an increase in toughness (Gormley *et al.*,1993) in the case of fish. The current study investigated the sous vide cooking of cod and salmon portions followed by freezing or chilling, and involved a range of physico-chemical and sensory tests to determine acceptability and product quality.

Materials and Methods

Ideal texture: Batches (2-3 kg in ca 500 g portions) of cod and salmon were simmered in water at 90^0C and samples were removed on seven occasions at intervals over a period of 50 min. These were subjected to immediate sensory analysis and four trained panelists were asked to mark the texture score on a 6 cm line with end-points too soft (0) to too firm (6) with the mid point (3) the ideal texture. Duplicate samples were sheared hot and cold (24 h at 4^0C) using a Kramer shear press fitted with a standard shear test cell (100 g samples). In this way the shear values equivalent to the ideal texture as sensed by the panelists were obtained, and became the target textures for delivery by the sous vide cooking process.

Sous vide cooking: Fresh and previously frozen (blast at -35^0C for 2 h; stored at -25^0C for 1 w; thawed at 4^0C overnight) portions (ca 160 g) of cod and salmon (i.e. 2 pre-treatments) were packed in sous vide bags, vacuumised, and cooked in a Barriquand Steriflow cooker using short (11 min at 80^0C) or long (105 min at 80^0C) cook times. These delivered pasteurisation (P) values of $P_{70} > 2$ min and $P_{90} > 10$ min respectively. The cooked samples were stored at 4^0C or were blast frozen at -35^0C followed by storage at -25^0C for 1 w (i.e. 2 post sous vide treatments).

Quality and sensory tests: The cod and salmon sous vide cooked samples were subjected to the following tests: gravitational drip, centrifugal drip, moisture content, colour (Hunter Lab), and Kramer shear tests (100 g samples) as described previously (Tansey et al., *2002*); thiamine content (Tansey *et al*., *2002*), TBARS (in the case of salmon); and paired comparison taste panels (20 tasters; preference) (see Results and Discussion for details) on fish cooked using the short cook time.

Results and Discussion

The results are presented for the main factors/effects (i.e. the pre-treatments, the cook times, the post sous vide treatments) since analysis of variance showed no statistically significant interactions between the factors.

Ideal texture: This was in the range 2.4 – 4.2 kN (cod) and 1.9-2.2 kN (salmon) (cold shears) indicating that ideal cod texture was firmer than ideal salmon texture; this may be due to the different mouthfeel between the species because of the much higher fat content of the salmon. The ideal texture of the species as sensed by the taste panel was maintained over a long simmering time, i.e. texture changed very slowly during cooking (also confirmed by the shear values). This contrasts with the texture of other products, such as broccoli, which softens rapidly with simmering time.

Effect of pre-treatments: Using raw vs previously frozen fish for sous vide cooking had a minimal effect on all the quality parameters of the sous vide cooked samples with the exception of gravity drip for sous vide cooked salmon where raw starting material (12.7%) gave a higher ($P < 0.05$) drip in the sous vide pack than previously frozen material (11.1%); this may be due to the latter losing some drip on thawing before insertion into the sous vide bags.

Effect of sous vide cook time: The short cook time gave a lower gravity drip loss (both species), a redder salmon colour, and a lighter cod colour (Table 1) than the long cook time. However, in the case of shear values the short cook time gave the firmest flesh in cod but the softest in salmon. Cook time did not influence centrifugal drip values, moisture content of either species, or the TBARS values in the case of salmon. The long cook times gave a lower thiamine content in cod and a higher value in salmon (Table 1) but the effects were small in practical terms.

Table 1: Effect of short (11 min/80^{0}C) vs long (104 min/80^{0}C) sous vide cook times on some quality parameters of cod and salmon

	Cook time for cod			Cook time for salmon		
Parameter	**Short**	**Long**	**F-test**	**Short**	**Long**	**F-test**
Gravity drip (%)[1]	20.2	22.4	P<0.05	8.9	14.9	P<0.001
Redness (a)[2]	-	-	-	12.9	10.9	P<0.05
White/yellow ratio (L/b)[2]	9.9	8.2	P<0.001	-	-	-
Shear (kN)[3]	1.82	1.69	NS	1.36	1.78	P<0.001
Thiamine (µg/1000g)	0.07	0.06	P<0.01	0.17	0.18	P<0.001

[1](W/W); [2]Hunter Lab values; [3]100 g samples in Kramer test cell

Effect of freezing vs chilling post sous vide cooking: Freezing post sous vide cooking gave a lower gravity drip loss (both species), a higher centrifugal drip loss (both species), less white cod flesh, less red salmon flesh, and a firmer texture (both species) (Table 2). These findings were expected (with the exception of gravity drip loss) as freezing causes loss of red pigment in salmon and a toughening of texture (Gormley *et al.*, 1993). Freezing vs chilling post sous vide cooking had no influence on moisture content (both species), or rancidity (TBARS) in the case of salmon. Freezing post sous vide cooking gave better retention of thiamine than chilling for salmon but this effect did not occur for cod (Table 2).

Table 2: Effect of freezing vs chilling post sous vide cooking on some quality parameters of cod and salmon

	Cod			Salmon		
Parameter	**Frozen**	**Chilled**	**F-test**	**Frozen**	**Chilled**	**F-test**
Gravity drip (%)[1]	20.1	22.5	P<0.05	11.2	12.3	P<0.05
Centrifugal drip (%)[1]	17.4	11.5	P<0.01	16.3	14.1	P<0.01
Whiteness (L)[2]	72.6	74.7	P<0.01	-	-	-
White/red (L/a)[2]	-	-	-	6.1	5.1	P<0.05
Shear (kN)[3]	1.94	1.58	P<0.001	1.78	1.36	P<0.001
Thiamine (µg/1000g)	0.06	0.06	NS	0.19	0.17	P<0.001

[1](W/W); [2]Hunter Lab values; [3]100 g samples in Kramer test cell

Paired comparison sensory tests: Freezing vs chilling post sous vide cooking had no statistically significant effect on taste panel response (20 tasters) for cod (7:13 preference ratio) or salmon (11:9 preference ratio). However, double freezing (i.e. using previously frozen/thawed fish for sous vide cooking followed by freezing post-sous vide cooking) had a deleterious effect in salmon (4:16 preference ratio; $P < 0.01$) in comparison with single freezing using fresh (i.e. not previously frozen) fish for sous vide cooking. However, this was not observed for cod (11:9 preference ratio).

Conclusion

These tests indicate that sous vide/freezing is a suitable technology for cod and salmon portions.

Acknowledgements

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P 7. NEW TECHNOLOGY REMOVES PIN BONES IN WHITEFISH

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Abstract

The industry has long had fillet machines that remove backbones and skin from the fish, but until today, manual clean cutting of fillets has been necessary to remove pin bones in whitefish, such as cod. Now, those bones will be removed as they pass through a pin bone removal machine, and thereafter each fillet will be controlled by x-ray. Automatic sorting will ensure that fillets with bone remnants are removed from the production line for manual processing. The remnants will be clearly marked as red lines on the x-rays, and the Inspector can see exactly where they are and quickly remove them. The goal is that at least 70% of the fillets will be boneless as they exit the pin bone removal machine while the remaining 30% can have remnants that must be manually removed. The new technology means that the number of employees in this part of production can be more than halved, greatly reducing the wage costs involved with manual fillet cutting. Simultaneously as there will be far less hard physical work, the personnel can be freed for other tasks.

The main economic profit will be achieved because more of the best fillet meat can be used for the best-paid fish products. While manual removal of pin bones involves removing some of the loin, the mechanical process ensures that primarily the bones are removed. With automatic removal, 4-6% more fillet will remain in production of cod and saithe. The variation is due to the properties of the raw material, which depend on various conditions, e.g. time from catch to processing. The new technology is developed in close collaboration between the equipment manufacturers Icelandic Marel and Danish Carnitech, the Norwegian fish processing industry, and Norwegian research institutes. Fiskeriforskning has led and co-ordinated the Norwegian research efforts.

Results and Discussion

The project was divided into two parts; (1) Development of a pin bone remover for whitefish and (2) development of an X-Ray based detection unit for inspection purposes. Part one mainly involved Carnitech, while part two mainly involved Marel, Norut IT and Fiskeriforskning. The outcome of the project is two separate machines that can be put into a production line as outlined in Figure 1. After post trimming fillets should be without bones, parasites, skin remnants and blood spots, and ready for final processing and packaging.

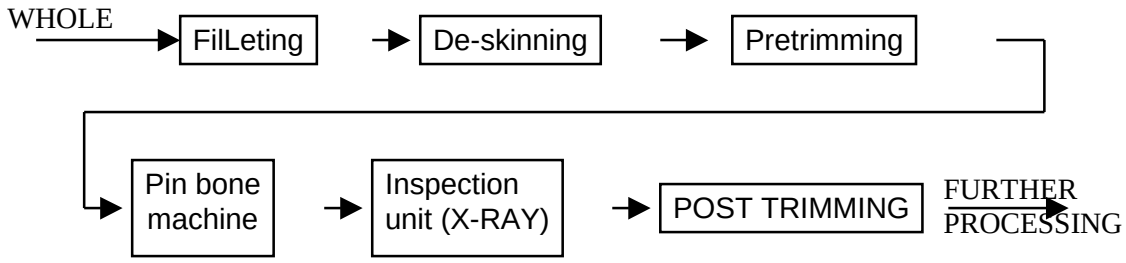

Figure 1. A possible production line that includes a pin bone remover and an inspection unit

The pin bone removal unit is significantly different from corresponding equipment developed for salmon. This is due to the way pin bones are positioned in whitefish compared to red fish. A traditional pin bone remover for red fish would remove too much fish flesh in order to remove pin bones in white fish. One of the key features with the new pin bone remover is how the pin bones are exposed to the picking device, see Figure 2.

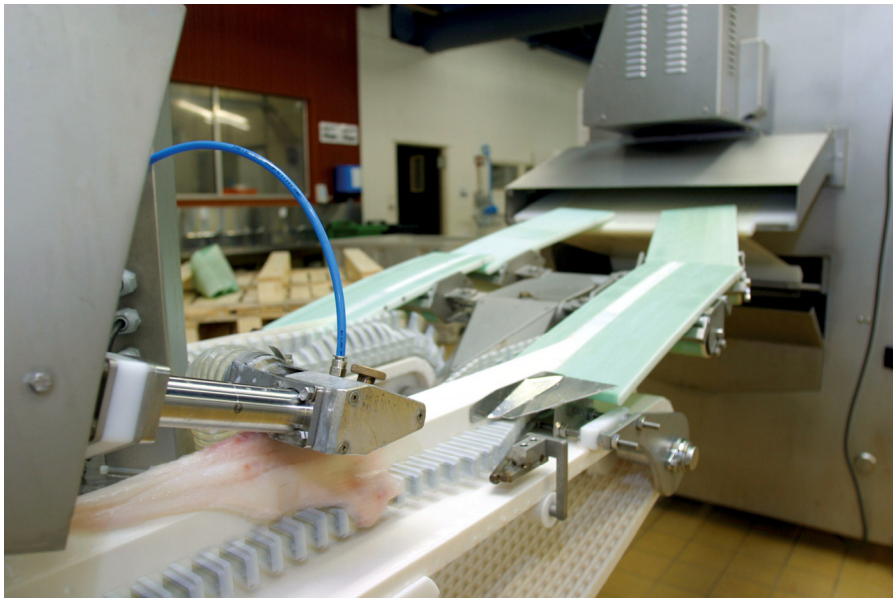

Figure 2. The pin bone removal machine operating on a cod fillet.

In the X-Ray unit, where all fillets are inspected, images are created and analysed to identify remaining bones. This will include pin bones, but also all other types of bones like for instance fin bones. A software solution is developed that automatically analyse the images, and if bones are present send a signal to the post trimming station. One of the challenges is that approximately one second is available for processing of each fillet. Fillets with bones are removed from the float line for final trimming, and the trimmer is presented an image of the fillet where bones are marked with red colour, see Figure 2. This simplifies the final cutting operation that has to be performed manually. In a future perspective this manual operation hopefully can be automated as well.

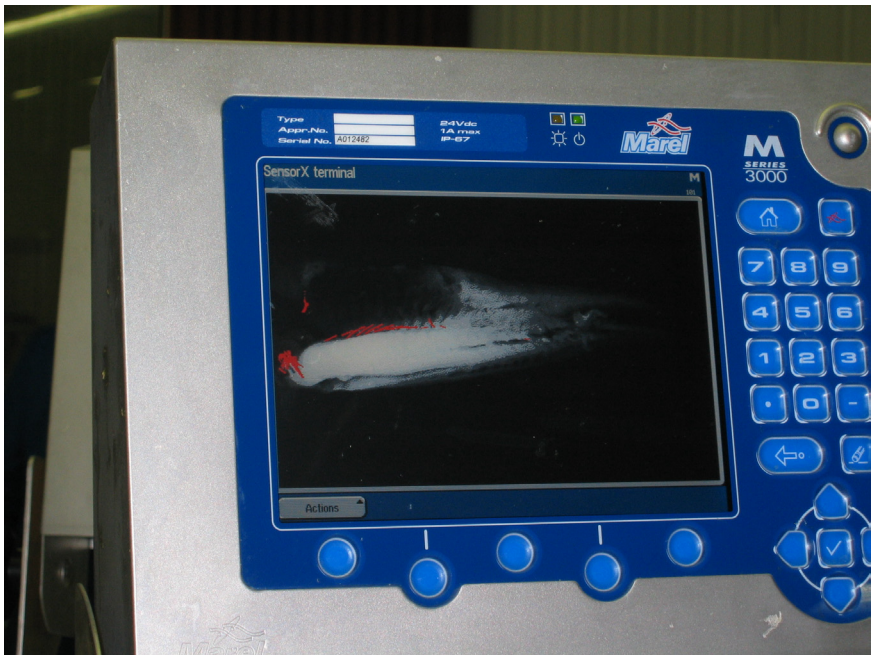

Figure 3. Visualisation of remaining bones in fillet as presented for post trimming. Bone remnants are clearly marked with red colour in image.

Conclusions

From a time limited project that has involved equipment manufacturers, fish processing industry and research institutes a large leap for the fish processing industry has been accomplished. With this new technology more profit is ensured for the fish processors, and for the consumers, bone free fish products should be available in the future.

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P 8. Effect of temperature and storage condition on the spoilage of herring

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Abstract

The aim of Nordic Industrial Fund Project "Optimal storage technology on board purse seiners" is to develop new storage technology that is optimised regarding fish quality, energy, investments and environment.
In an experiment we simulated herring stored in bulk and in chilled sea water by storing it in sealed plastic bags. The bags were stored at 0, 3.7 and 9.5 °C, and the content of one bag was used for analyses. In the bulk experiment, the formation of TMA are described by a sigmoid functions, while ammonia, TVB-N – TMA-N, are described by polynoms. The activation energy for TMA-formation in herring stored in bulk is calculated to 108 kJ/mol.

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P 9. CHEMICAL CHANGES RELATED TO QUALITY LOSS DURING FARMED TURBOT (*Psetta maxima*) CHILLING BY APPLYING FLOW AND TRADITIONAL ICING

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Abstract

The present work focuses on the comparative analysis of the loss of quality underwent by farmed turbot during chilled storage either in traditional ice or in flow ice. To this end, the evaluation of changes in the nucleotides ─as determined by the K value─, lipids ─as determined by the investigation of free fatty acids, peroxide value and, thiobarbituric acid index ─, and proteins ─basically modifications of either solubility and electrophoretic profiles─ throughout a long storage period ─40 days─ was carried out in parallel to sensory analysis.

According to the results obtained, the employment of flow ice for the storage of farmed turbot is encouraged, due to the better maintenance of quality and extended shelf life derived from its use.

Introduction

Seafood products have attracted considerable attention as a source of high amounts of important nutritional components to the human diet. However, in recent years the fishing sector has suffered from dwindling stocks of traditional species as a result of dramatic changes in their availability, a fact that has prompted fish technologists and the fish trade to pay more attention to aquaculture techniques as a source of fish and other seafood products (FAO, 2000; Josupeit et al., 2001). Turbot (*Psetta maxima*, also known as *Scophthalmus maximus*) is a flat fish species of high commercial value found in Northern waters. In recent years, the increasing production of this species as an aquaculture product has made it more available to consumers.

Assurance of both seafood quality and safety will be a major challenge to be faced by humankind in this new century. In this sense, wild and farmed fish species are known to deteriorate rapidly after death due to the action of different mechanisms (Pigott and Tucker, 1987). Thus, and although an extensive work has been carried out on the investigation of the effects of fish diets on the growth and the lipid composition of turbot (Regost et al., 2001), the elucidation of the damage mechanisms occurring in farmed turbot during chilled storage remains, up to now, relatively unknown.

Liquid ice offers a promising technique for preservation and consists of an ice-water suspension at a subzero temperature. Two main features of liquid ice are its faster chilling rate and the reduced physical damage caused to seafood products by its microscopic spherical particles, as compared with traditional ice. Despite its theoretical advantages, few empirical data concerning the practical advantages derived from the use of liquid ice for the storage of marine species are available (Huidobro et al., 2002).

With the aim of gaining deeper knowledge about the biochemical changes occurring during chilling storage of farmed turbot species, in the present work we have compared the results obtained for sensory and chemical parameters in specimens maintained in traditional ice and liquid ice.

Materials and Methods

In this work, a liquid-ice prototype (Kinarca S.A.U., Vigo, Spain) was used. The composition of the liquid-ice binary mixture was 40% ice/60% water, prepared from filtered seawater. Two-year old farmed turbot (*Psetta maxima*) specimens were obtained from the Stolt Sea Farm, S.A. (Carnota, Galicia, Spain). Fish specimens were sacrificed in a water-ice mixture and then kept in ice for 10 h as they arrived at our laboratory. Samples were taken for analysis on days 0, 2, 5, 9, 14, 19, 22, 26, 29, 33, 36 and 40. Three different batches for each ice technology were considered and studied separately along the whole experiment. The sensory analyses were conducted by a taste panel consisting of five experienced judges, according to traditional guidelines concerning fresh and chilled fish (DOCE, 1989)

The lipid fraction was extracted using the Bligh and Dyer method.(1959) The thiobarbituric acid index (TBA-i) was determined according to Vyncke (1970) using a 5% trichloracetic acid extract of fish muscle. Free fatty acids (FFA) content was determined by the Lowry and Tinsley (1976) method based on complex formation with cupric acetate-pyridine. Results were expressed as g FFA/100 g lipids. Peroxide value (PV) ─expressed as milliequivalents (meq) of oxygen per kg of lipids─ was determined according to the ferric thiocyanate method (Chapman and McKay, 1949).

Sarcoplasmic protein extracts were prepared in a low-ionic-strength buffer composed of 10 mM Tris-HCl, pH 7.2 + 50 mM PMSF. Protein concentrations in the extracts were determined by means of the protein microassay method (Bio-Rad Laboratories Inc. Hercules, CA). Electrophoretic analysis was carried out in horizontal commercial SDS-PAGE gels. (Excel-Gel SDS Homogeneous 15%, Amersham Biosciences, Uppsala, Sweden) according to Piñeiro et al. (1999). Gels were silver stained.

Data from the different chemical measurements were subjected to one-way analysis of variance; comparison of means was performed using a least-squares difference (LSD) method (Statsoft et al , 1994).

Results and Discussion

According to the results of the sensory analyses, the turbot specimens stored in liquid ice maintained good quality, being classified in the E or A categories up to day 22. However although farmed turbot stored in conventional ice has a longer shelf life than other common medium-sized fish species, the storage of turbot in liquid ice allowed a significant extension of its shelf life, from 14 days to 22 days.

In reference to nucleotides measurements, the K values of the turbot specimens stored in liquid ice proved to be considerably lower than those described for turbot stored in conventional ice: in the latter case, the K values reported levels higher than 50% and 70% after 9 days and 14 days of storage, respectively.

In comparison with the initial fish material at day 0, a significant increase in the free fatty acids content was observed on day 14. The rate of lipid hydrolysis in the liquid ice batch proved to be quite similar to that observed in turbot stored in conventional ice, the latter also showing FFA concentrations below 2.5% even after 40 days of storage. As expected from the results obtained, statistical analysis confirmed that liquid ice did not allow any significant reduction in the rate of lipid hydrolysis in turbot muscle ($p<0.05$) as compared with conventional ice.

Lipid oxidation was investigated by means of the TBA-i. The formation of thiobarbituric acid-reactive substances (TBARS) showed few variations up to day 26. After this, a sharp increase was observed on day 29, followed by minor variations up to the end of the storage period. TBARS formation was found to be also low in turbot muscle stored in conventional ice.

With respect to the results of protein extractability from turbot muscle in the low ionic-strength buffer used, a significantly high extractability was observed at all sampling times, even in advanced periods of storage in liquid ice. These results are clearly higher than the extractability values obtained for turbot muscle when storage was carried out in conventional ice. Moreover, a better stabilization of the protein fraction above 94 kDa was achieved in turbot muscle subjected to storage in liquid ice as compared with conventional ice.

Compared to traditional icing, storage of turbot in flow ice implied a partial inhibition of both the nucleotide degradation pathway ─as determined by the K value─, and lipid oxidation mechanisms ─as determined by measurement of fluorescent compounds─. Besides, a higher stability of high-molecular weight proteins was also observed throughout storage in the flow ice batch. Chemical changes correlated well with respect to sensory evaluation, and both analyses indicated a shelf life of 20 days for turbot stored in traditional ice, while an extended shelf life ─30 days─ was determined for turbot stored in flow ice.

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P 10. EFFECT OF CHILLED STORAGE IN FLOW ICE ON THE MICROBIAL QUALITY AND SHELF LIFE OF FARMED TURBOT (*PSETTA MAXIMA*). ISOLATION AND IDENTIFICATION OF MAJOR PROTEOLYTIC BACTERIA

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Abstract

The application of flow ice (FI) at subzero temperature as a new preservation method of farmed turbot (*Psetta maxima*) ─a flat fish species with an increasing commercial interest─ is discussed. For it, comparative sensory and microbiological analyses were carried out in batches of farmed turbot stored in FI or in traditional ice (TI) up to 40 days. Statistically-significant differences ($p<0.05$) according to the Scheffé test were determined between the FI and TI batches for aerobic mesophiles, anaerobes, coliforms, proteolytic bacteria and pH, the FI batch showing lower microbial numbers and lower pH values in all cases. Moreover, significantly lower concentrations of trimethylamine and total volatile bases were also determined in the FI batch than in the TI batch. According to the microbial and sensory analysis, the quality of farmed turbot stored in TI remained high until day 14th, while this period was extended to 22 days in the batch stored in FI.

Lactic acid bacteria (LAB), mainly *Lactobacillus delbrueckii* subsp. *delbrueckii* and *Lactococcus lactis* subsp. *lactis* strains ─many of them showing clonal heterogeneity in their phenotypes─ were predominant among the proteolytic strains isolated from chilled turbot stored in TI and FI, respectively. Proteolytic strains of *Aeromonas salmonicida, Brevundimonas vesicularis, Vibrio parahaemolyticus* and *Weisella viridescens* were also recovered from chilled turbot at lower rates, while proteolytic strains of *L. lactis* subsp. *lactis, Brochothrix thermosphacta, Plesiomonas shigelloides, Proteus vulgaris* and *Pantoea* sp. were predominant in turbot specimens subjected to abusing temperatures. The observed low bacterial growth together with the predominance of proteolytic LAB over proteolytic Gram (─) microorganisms may be related to the extended maintenance of the quality and shelf life of farmed turbot.

Introduction

Flow ice (FI) offers a promising technique for preservation and consists of an ice-water suspension at a subzero temperature. Two main features of FI are its faster chilling rate –deriving from its higher heat-exchange capacity– and the reduced physical damage caused to seafood products by its microscopic spherical particles, as compared with traditional ice (TI). The overall covering of the fish surface by the FI mixture also protects the fish from the action of oxygen. The versatility of the FI technique has also been reported to be good. Thus, its pumpability and potential combination with other additives –such as ozone or melanosis inhibitors– are especially remarkable (1). However, despite its theoretical advantages, few empirical data concerning the practical advantages derived from the use of FI for the storage of marine species are available. With the aim of gaining deeper knowledge about the potential applications of FI technology, in the present work the effect of this advanced storage system on quality losses and the shelf life of farmed turbot was evaluated. Turbot (*Psetta maxima*), is a highly valued flat fish species appreciated for its firm, white and flavourful flesh. Recently, increasing production of this species as an aquaculture product has raised its availability. To this end, the evolution of the sensory and microbiological features was evaluated in farmed turbot stored in FI for a long storage period (44 days). The results were compared with previous studies addressing the use of TI (2, 3).

Materials and Methods

A FI prototype (Kinarca S.A.U., Vigo, Spain) was used. The composition of the FI binary mixture was 40% ice/60% water, prepared from filtered seawater. The temperature of the FI mixture was -2ºC. Two-year old farmed turbot specimens were sacrificed in a water-ice mixture, surrounded by FI at a fish/FI ratio of 1/1, and stored for up to 44 days in an isothermal room at 2ºC. A control batch was stored in TI. Samples were taken from each batch on days 0, 2, 5, 9, 14, 19, 22, 26, 29, 33, 36, 40 and 44. Sensory analysis was conducted by a taste panel consisting of five experienced judges, according to traditional guidelines concerning fresh and chilled fish (4). Sensory assessment of the fish included the following parameters: skin, external odour, gills, consistency and flesh odour. Microbiological analyses included the investigation of aerobic mesophiles, anaerobes, coliforms and proteolytic

bacteria. The identification of proteolytic strains was accomplished using miniaturized biochemical tests. Total volatile base-nitrogen (TVB-N) values were measured as described elsewhere (5). Trimethylamine-nitrogen (TMA-N) values were obtained by the picrate method, as previously described (6).

Results and Discussion

Turbot specimens stored in FI maintained good sensory quality, being classified in the E or A categories up to day 22. After this time, quality decreased, and by day 33 the turbot stored in FI was no longer acceptable. In contrast with these results, the good sensory quality –E and A categories– of farmed turbot specimens stored in TI were reported to be maintained only up to day 14, such turbot specimens being rejected on day 22 (3). In all cases, the TVB-N content of turbot stored in FI was very low, statistical analysis revealing that the storage of turbot in FI involved a significantly ($p<0.05$) slower formation of TVB-N in comparison with storage in TI. Average TMA-N values below 0.4 mg/100 g muscle and 0.5 mg/100 g muscle were obtained for turbot stored in FI on days 22 and 26 of storage, respectively. In contrast, average levels above 1.1 mg/100 g muscle and 1.8 mg/100 mg muscle have been reported for turbot muscle stored for 22 days and 26 days in TI, respectively (3). As expected from the results obtained in the present study, the formation of TMA-N in turbot stored in FI was significantly ($p<0.05$) lower than in turbot stored in TI.

The concentrations of aerobic mesophiles reached levels only slightly above 10^4 CFU/g even after 36 days of storage in FI. A previous report about turbot stored in TI reported concentrations of aerobic mesophiles above 10^6 CFU/g after 14 days of storage (3). The levels of proteolytic bacteria in turbot muscle neither reached levels of 10^5 CFU/g until very advanced storage periods in FI (40 days). By contrast, a previous study on turbot stored in TI reported significant ($p<0.05$) changes in the concentrations of proteolytic bacteria after only 14 days of storage, these concentrations reaching levels of $7.5x10^6$ CFU/g at that time (3). The concentrations of anaerobes in turbot muscle stored in FI did not vary significantly ($p<0.05$) until day 44 of storage. Up to that moment, the levels of this microbial group were in all cases below 10^3 CFU/g . These results clearly indicate the very slow growth of this microbial group in turbot muscle stored in FI. In contrast to the results obtained for FI, the levels of anaerobes in turbot muscle stored in TI have been reported to vary significantly ($p<0.05$) after 26 days of storage, reaching levels above $3.8x10^4$ CFU/g at that time (3). With respect to the development of coliforms in turbot muscle stored in FI, these did not vary significantly along the 44 days of storage. Thus, it is striking that the average levels of coliforms were below 10 CFU/g even after 36 days of storage. In contrast, a previous report on turbot muscle stored in TI indicated a significant ($p<0.05$) increase in the coliform concentrations after 22 days of storage, reaching levels close to 10^4 CFU/g after 29 days of storage (3). According to the Tukey and Scheffé tests, the results obtained indicated statistically-significant differences ($p<0.05$) between the FI batch and the TI batch for all four microbial groups. These findings clearly indicate a significantly slower growth of the four microbial groups investigated in turbot muscle subjected to storage in FI as compared with TI. These results correlate well with the differences observed in the sensory studies, which indicated a better maintenance of sensory quality in turbot stored in FI.

Ten of the 14 proteolytic bacteria isolated from the FI batch belonged to the species *Lactococcus lactis* subsp. *lactis*. Another lactic acid bacteria (LAB) strain –*Lactobacillus paracasei* subsp. *paracasei*– was also successfully identified. The other three isolates corresponded to the species *Aeromonas salmonicida*, *Brevundimonas vesicularis* and *Vibrio parahaemolyticus.* It should be mentioned that the predominant isolate obtained from turbot muscle subjected to abusive temperatures was a *L. Lactis* subsp. *lactis* strain. A previous study carried out at our laboratory reported the isolation of other homofermentative LAB strains from turbot stored in TI, *Lactobacillus delbrueckii* subsp. *delbrueckii* being the predominant species (3). In contrast to the results obtained in this work, other bacteria such as *Plesiomonas shigelloides*, *Brochothrix thermosphacta* or *Proteus vulgaris* have been reported to predominate in turbot stored in TI and then subjected to abusive temperature (3).

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P 11. THE SURVIVAL OF *SALMONELLA* AS AFFECTED BY CHITOSAN EXTRACT FROM CRAB SHELL

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Abstract

Salmonella can be found in any raw food of animal origin, as well as some fruits and vegetables. It is the most frequently reported cause of foodborne illness, Salmonellosis, which can be life-threatening especially for persons with impaired immune systems. Chitosan, a natural antibacterial agent derived from chitin found in the exoskeleton of crab, was used to determine the inhibition of *Salmonella* spp. growth in medium. Three levels of chitosan, 1 %, 2% and 3 %, were prepared in 0.5% acetic acid. The pH was adjusted to 6.5 using 1 N NaOH. Pure cultures of *Salmonella* spp. (6.0 x 10^8 CFU/ml) were inoculated into (a) nutrient broth alone (control), (b) nutrient broth containing 1%, 2%, 3% of chitosan, or (c) 0.5 % acetic acid. All cultures were kept at 7 °C throughout the entire experimental period. Inoculated nutrient broths were consecutively removed for bacterial examination at a 12-hr interval for a period of 48 h. Results indicated that a 7-log reduction was observed after 48 h incubation at 7 °C regardless of levels of chitosan. However, 0.5% acetic acid alone showed a 2-3 log reduction. For the mixed culture recovered from egg shell, an 8-log reduction of total plate count was observed after 24 h incubation. This study implied that 1 % chitosan solution can be used as an antimicrobial agent to ensure the safe of foods.

Introduction

Raw meat, poultry, eggs, dairy products, and some fruits and vegetables may carry *Salmonella* bacteria. *Salmonella* can survive to cause illness if these foods are not thoroughly cooked. Although not all cases of foodborne illness are reported, it has been estimated that anywhere from 696,000 to 3.8 million people contract salmonellosis each year (1). According to CDC, most people experience diarrhea and abdominal camps within 8 to 72 hours after the contaminated food was eaten. The elderly, infants, and those with impaired immune systems are more likely to have a severe illness (5). Because preservatives are very important to maintain the quality of food and inhibit the growth of various spoilage and pathogenic bacteria. It is desirable that some new, non-toxic natural preservatives be studied and developed in order to replace artificial preservatives. Chitosan, a modified carbohydrate polymer derived from chitin found in the exoskeleton of shellfish, especially crab, is known for its biodegradability, biocompatibility and the potential for modification. Therefore, uses of chitosan and their derivatives to inhibit the growth of microorganisms have received considerable attention in recent years. The objective of this study was to determine the antimicrobial ability with various levels of chitosan on *Salmonella* spp. and microflora found on the egg shell.

Materials and Methods

Preparation of chitosan solutions

Commercilal chitosan (Source Naturals, INC. Scotts Valley, CA.) at 1 %, 2%, or 3% (w/v) were added to 0.5% acetic acid. The pH value was adjusted to 6.5 by using 1 N NaOH.

Preparation of Cultures

***Salmonella* spp. :** *Salmonella* spp. was isolated from the chicken breast and used as a target bacterium. The tested *Salmonella*, maintained on PCA slant, was transferred to a new PCA slant every 2 weeks and kept at 7 °C until to be used. *Salmonella* was aseptically subcultured into nutrient broth and incubated at 37 °C for 24 h. The pure *Salmonella* (6.0 X 10^8 CFU/g) culture was inoculated in following media : (a) nutrient broth alone, (b) nutrient broths with 1%, 2%, and 3% of chitosan addition, and (c) 0.5 % acetic acid. All tubes were kept at 7 °C and consecutively removed for bacterial examination at a 12-hr interval for a period of 48 h.

Microflora from egg shell

The unwashed chicken eggs, obtained from Poultry Research Center, The University of Georgia, Athens, GA., were soaked in peptone water for 1 h. One ml of the peptone water was transferred to tubes with 9 ml nutrient broth. After 24 h of incubation at 37 °C, the population reached 4.3 X 10^9 CFU/ml and inoculated in nutrient broth in the same condition as pure *Salmonella* spp culture. Incubated nutrient broth tubes were consecutively removed for bacterial examination at a 6-hr interval for a period of 24 h.

Counting technique

The tubes with broth were gentlely agitated for 15 sec. and then 1-ml portion from each tube was appropriately diluted with peptone broth dilution to 10^{-7}. One ml of each dilution was transferred onto petri dishes

and then covered with plate count agar using pour plate technique. Plates were incubated at 37 °C for 24 h and colony forming units were counted.

Results and discussion

Table 1. Antimicrobial effect of chitosan on *Salmonella* spp. culture

	Salmonella spp. (CFU/ml) population				
Time(h)	Control	Chitosan 1%	Chitosan 2%	Chitosan 3%	Acetic acid 0.5%
0	6.0×10^8	6.0×10^8	6.0×10^8	6.0×10^8	6.0×10^8
12	6.5×10^8	3.2×10^7	8.6×10^6	2.3×10^6	2.5×10^8
24	8.8×10^8	6.7×10^5	8.2×10^4	5.8×10^4	9.4×10^7
36	3.5×10^9	8.6×10^3	4.3×10^3	4.0×10^3	6.9×10^6
48	3.9×10^9	<100	<100	<100	3.5×10^6

The effect of all 3 levels of chitosan on the reduction of *Salmonella* spp. on medium at pH 6.5 is shown in Table 1. Compared to the control, growth of *Salmonella* spp. was apparently inhibited by various levels of chitosan at 1 % and higher. The population of *Salmonella* spp. was reduced to less than 100 CFU/ml, with a 7-log reduction observed after 48 h, regardless of the incubation level of chitosan. However, *Salmonella* spp. in 0.5% acetic acid alone showed only a 2-3 log reduction.

Table 2. Antimicrobial effect of chitosan on total plate count of egg shell

	Total plate count (CFU/ml)				
Time (h)	Control	Chitosan 1%	Chitosan 2%	Chitosan 3%	Acetic acid 0.5%
0	4.3×10^9	4.3×10^9	4.3×10^9	4.3×10^9	4.3×10^9
6	5.2×10^9	5.2×10^8	3.7×10^8	3.2×10^8	2×10^9
12	6.0×10^9	4.5×10^6	8.8×10^5	6.5×10^5	5.6×10^8
18	6.3×10^9	8.6×10^3	7.6×10^3	6.7×10^3	7.6×10^7
24	6.8×10^9	<100	<100	<100	6.5×10^6

Similar results were obtained for the total plate count on egg shell as shown in Table 2. The total plate count in all 3 levels of chitosan showed an 8-log reduction after 24 h incubation. However, total plate count in PCA with 0.5% acetic acid alone showed only a 2-3 log reduction; no reduction was found in control sample.

Chitosan generally showed stronger bactericidal effected with gram-positive bacteria than gram-negative bacteria in the presence of 0.1% chitosan. The minimum inhibitory concentration of chitosan was ranged from 0.05% to > 0.1% (4). Although the antibacterial activity of chitosan has been studied for more than 20 years, the exact mechanism of the antibacterial action of chitosan and their derivatives is still not known. There are different mechanisms have been proposed. The mode of antimicrobial action of chitosan on gram-negative bacteria such as *Salmonella* was studied with special emphasis on its ability to blind to and weaken the barrier function of the outer membrane (2). Interaction between positively charged chitosan molecules and negatively charged microbial cell membrane leads to the leakage of proteinaous and other intracellular constituents, or due to the water-binding capacity and inhibition of various enzyme. Chitosan also has bioabsorbant activity and can absorb nutrients of bacteria and may inhibit their growth (3).

Conclusion

This study provided some information about the efficacy of chitosan on *Salmonella* spp.and total plate count on egg surface. The data implied that 1% chitosan can be used as antibacterial agent to ensure the safe of food. Many factors such as the character of chitosan used and condition of food to be applied should be considered as the influence the effectiveness of chitosan as an inhibitor.

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Developement of fish products for health and well-being.

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First Joint Trans-Atlantic Fisheries Technology Conference - TAFT 2003
33th WEFTA and 48th AFTC meetings,
11-14 June 2003, Reykjavik - Iceland

K 2. DEVELOPMENT OF NEW FUNCTIONAL FISH PRODUCTS TO PROMOTE HEALTH AND WELL BEING

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Abstract

The development and marketing of functional foods is a rapidly expanding area in the food industry. The most general definition of functional foods is that these foods are food made on the basis of knowledge of the nutrition-health relationship. The concept of functional foods started in Asia and was exported to western countries about 10-15 years ago. The concept appeared to be a trend rather than a hype, being supported by a variety of driving forces, including the desire of 55plus people to age healthy, increased knowledge of the nutrition-health relation ships, increased purchasing power of the population and the desire of the food industry to expand in added value products. The concept of functional foods offers opportunities to the food industry to improve existing foods and to design new foods with an added nutritional value. In fact two generations of functional foods can be distinguished: the first and second generation: products with nutrition claims and health claims respectively. Health claim may refer to reduction of risk of chronic disease or to enhancement of body functions.

Nutrients and other bioactive substance from fish as well as fish itself can be used as ingredients for functional foods. So there are established roles for vitamin D and calcium (bone health promotion), and for fatty acids from fish (reduction of risk of cardiovascular disease). Fatty acids from fish may also have a protective role in inflammatory diseases, weight management, bone health, the metabolic syndrome and colon cancer, but these issues need further scientific substantiation. Fish protein, because of its high digestibility, could have beneficial effects on the colon, by reducing protein fermentation. Also the preparation of bioactive peptides derived from fish protein could be further explored. Many more components from fish (including taurine, chitosan, glucosamine and phospholipids) gained interest as potential bioactive ingredients for functional foods, but the evidence for positive effects is limited and as yet largely insufficient for health claims.

Introduction

Since the introduction in the western world about 10-15 years ago of the concept of functional foods, the attention for the production and marketing of food with an added nutritional values has expanded rapidly. This development appeared to be a trend and not a hype. It was picked up rapidly in those sections of the food industry where a focus on health promotion already existed, e.g. the dairy industry and the margarine industry. The concept of functional foods in fact challenges the food industry to improve existing foods and to develop new foods, in such a way that these foods can promote health. After giving a brief overview of the general concept of functional foods, the relevant health areas and health claims, opportunities for functional fish products are described.

The concept of functional foods

Market developments and regulatory aspects

The interest of the food industry in the development and marketing of innovative foods with an added value is expanding rapidly. Interesting concept are those with an added value in health and/or convenience. So on the basis of a marketing survey, RABO bank International (2001) expects a worldwide 100 % growth of the market for functional foods in the next five years. At the same time consumers, their organizations and authorities become more critical with respect to the validity of health claims and it is recognized that there is a need for legislation at the European level of nutrition claims and health claims (DG Sanco, 2001). Additionally, not just critical questions about the validity of health claims, but also about the safety of functional foods and dietary supplements have to be answered. The novel food law in EU member states (Regulation No 258/97) implicates that the safety of novel foods - and thus also of functional foods as far as these are novel - has to be assessed before their marketing. Novel foods are defined as foods that hitherto have not been used for human consumption to a significant degree within the European Community. No specific legislation exists in European countries for functional foods. Although for health claims, no specific European legislation exists as yet, two basis rules, however, are in force: (1) consumers may not be misled and (2) medical claims for foods are not allowed. It is expected that European functional food legislation will allow reduced disease risk claims, provided scientific substantiation is adequate and approved. Over the world, and even among member states of the European Union, legislation with respect to health claims shows wide variations and further harmonization of EU food legislation is indeed required. The trends in food and health in human nutrition are also noticed in the pet food area, where companies, are looking for added value and discrimination power for their products.

Functional foods, two dimensions

There is no generally accepted definition for functional foods. In fact the characteristics of these foods (or drinks) are that they have been made on the basis of knowledge of nutrition and health and that they offer specific physiological benefits to the consumer beyond the basic nutritional value of existing (traditional) foods. Consumption of functional foods offers the opportunity to ingest a healthy balanced diet, with additional benefits, and the concept challenges the food industry to make existing foods better and to market new innovative foods. According to this concept, functional foods can be designed for the general population as well as for special target groups, like sports people, infants and patients. Two dimensions in the concept of functional foods can be recognized: traditional nutritional value and bioactivity beyond traditional nutritional value. The first dimension may include well-designed convenience foods, like meal replacing products (such as cereal breakfast bars and shakes and drinks developed in conjunction with weight loss programs) with a well-balanced nutritional composition and foods fortified with nutrients. The second dimension includes functional foods that exert effects which are beyond those of traditional nutritional value (Diplock et al., 1999) This type of functional foods refers to the supplementation or fortification with bioactive components, like prebiotics, probiotics, phytochemicals, bioactive peptides, long chain polyunsaturated fatty acids and herbals (such as fortified cereals, yoghurts and dairy drinks, cholesterol lowering spreads). Nutrition claims are often attached to products of the first category (dimension 1), whereas health claims are attached particularly to foods of the latter category (dimension 2). Fortification of foods with nutrients (vitamins, minerals, trace elements, essential fatty acids and amino acids) can serve both dimensions, depending on the concentrations used. When nutrient concentrations in foods are such that a daily portion of these foods makes a significant contribution to Daily Recommended Intakes, they can be considered as offering traditional nutritional value. Nutrient concentrations in excess of traditional recommendations could serve the second dimension of functional foods.

Dietary supplements, nutriceuticals, nutraceuticals

According to the Dietary Supplement Health and Education Act (1994) in the USA, dietary supplements are products intended to supplement the diet to enhance health. Dietary supplements appear on the market as pills, capsules, powders, or drinks. They are also called nutriceuticals or nutraceuticals. The legal status of dietary supplements may vary among countries. In most European countries dietary supplements fall under food legislation. Only some EU countries - for example Germany and Denmark- tend to view dietary supplements as pharmaceuticals, and Finland views dietary supplements distinctly from food, although not as pharmaceuticals. .In some other countries, these products are considered legally as a distinct category of foods, for example in the USA they fall under the Dietary Supplement Health and Education Act (1994). Nutriceuticals (primary nutrients) can be considered as dietary supplements containing essential nutrients or mixtures thereof (vitamins, minerals, trace elements) and nutraceuticals (secondary nutrients) as dietary supplements containing other types of bioactive substances. This discrimination between nutri- and nutraceutcals is quite relevant, because for essential nutrients there is no doubt about bioactivity, whereas for many nutraceuticals this activity still has to be demonstrated.

Application fields for functional foods

Indicated in Table 1 are the application fields for functional foods and dietary supplements (prevention of nutrition-related diseases and enhancement of body functions).

Table 1. Fields for application of functional foods

Diseases (Reduced risk of disease claims)	Body functions (Enhanced function claims)
Obesity	
Cardiovascular disease	Natural defense
Hypertension	Bowel function/ gut health
Hyperlipidaemia	Mental performance
Diabetes type II	Physical performance
Osteoporosis	Oral health
Allergy	Skin health
Cancer	
Inflammatory diseases	
Coeliac disease	

Undoubtedly the major health problem in western societies is the increasing prevalence of overweight and obesity. In the Netherlands for instance the number of overweight adults (Body Mass Index higher that 25) increased from 30% in 1980- to more that 45% in 2000. In the same period the number of obese adults raised from 5 to more than 9 % now. In the United States these figures are even worse: more that 55% of the adult population has overweight and more than 20% is obese. Health authorities are particularly concerned about the increasing prevalence of overweight and obesity in children. So in the Netherlands 9% of the children had overweight in 1980, now the figure is more than 14%. Overweight and obesity are associated with an increased risk of diabetes

type II, with hyperlipidaemia and with raised blood pressure. In fact a substantial part of the population in western countries displays the metabolic syndrome, also called syndrome X. It is a combination of overweight (abdominal fat), hypertension, elevated levels of blood lipids and insulin resistance and increases dramatically the risk for cardio vascular disease and diabetes type II.

Consumer acceptance of functional foods

Successful marketing of functional foods depends on the acceptance of consumers. Since functional foods should be consumed regularly to display the beneficial effect, the primary condition for acceptance is good taste. Three main other conditions for success are: efficacy, emotional fit and rational fit. Regarding efficacy, the product must be trusted by the consumer and this implies that the health claim connected with the product must be substantiated by scientific evidence. The product matrix should have an emotional and rational connection with the beneficial effect. So, for instance, consumers consider yogurt as a product that is easily digested and that promotes intestinal well being Yogurt is therefore a very good matrix for bioactive components that promote intestinal health, e.g. probiotics ans prebiotics. The first generation of the blood-cholesterol lowering diet margarines with high contents of polyunsaturated fatty acids is widely accepted and appeared a suitable matrix for the second generation of diet margarines, those with cholesterol-lowering phytosterols.

Frewer (2002) listed a number of additional critical issues, dealing with consumer acceptance of functional foods:

- Perception of a personal benefit
- Cultural differences
- Nutrition knowledge
- Quality of communication
- Perceived seriousness of disease
- Technology used (e.g. gmo)
- Health consciousness
- Benefit, nearby or distant
- Price
- Sensoric properties

Opportunities for fish

To investigate the opportunities for the fish industry to develop functional foods, it is useful to discuss the composition of fish in relation to its content of bioactive components. As indicated in Figure 1, fish as compared to meat, is a very rich source of a large variety of nutrients, especially protein, vitamin D, and vitamin B12. Figure 2 expresses the nutrient content as nutrient density. A density of 1 for a particular nutrient indicates that the product just contains enough of that nutrient in relation to its energy content to cover the recommended daily allowance for that particular nutrient. Not listed in Figure 1 is selenium, for which fish is also a good source. In addition, although normally not consumed, fish bones are a good source of calcium.

Fish is also a unique source of highly poly unsaturated n-3 fatty acids, e.g. EPA and DHA .

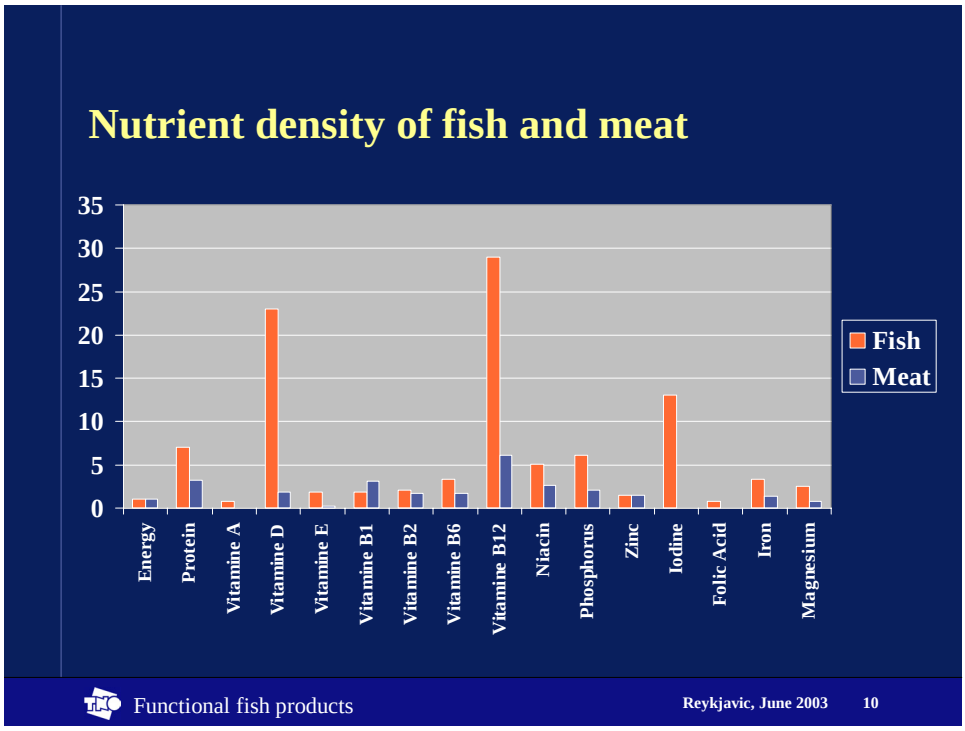

Figure 1. Nutrient composition of fish as compared to meat

Potential role of functional fish ingredients: n-3 fatty acids and fish proteins

A variety of fish components is applicable to one or more of the health promotion areas indicated above. In this regard we can mention calcium and vitamin D (osteoporosis prevention), and n-3 fatty acids (cardiovascular disease prevention). Also fish protein, because of its high digestibility, may have specific benefits for the colon. N-3 fatty acids may also play a significant role in diabetes type II prevention, reduction of inflammatory diseases and potentially in weight management. These areas should be explored further before any claims can be made. Therefore a proposal for an Intergrated Project on Seafood (SeafoodPlus) was submitted recently to the EU. A substantial part of this project proposal includes nutrition research on the significance of n-3 fatty acids and fish proteins, with the primary objective to investigate the potential role of fish consumption in lowering the risk of nutrition-related chronic diseases which have a high prevalence in western societies: cardiovascular disease, colon cancer, obesity, inflammatory bowel disease and osteoporosis. The second focus area is to assess the role of fish consumption in health promotion of young European populations, including pregnant women and their children. Therefore the research includes intervention studies, experimental studies and/or epidemiological studies on gastro-intestinal health, with special emphasis on reduction of risk of colon cancer and inflammatory bowel disease, gastro intestinal bioavailability of fish components with special emphasis on the development of stable, bio –accessible functional foods, health of young European populations, including pregnant women and their children and metabolism of n-3 fatty acids in relation to heart disease.

The major causes of premature morbidity and mortality in Europe are cardiovascular disease (c. 40%) and cancer (c. 25%). Recent epidemiological studies provide convincing evidence that fish consumption is related to improved health and reduced risk of chronic disease. This evidence is particularly strong for prevention of colorectal cancer and mixed in relation to prevention of cardiovascular disease and the metabolic syndrome. Increasing evidence supports an important role for long chain n-3 polyunsaturated fatty acids, such as those found in oil rich fish, in infant nutrition and brain development, in bone health and in appetite regulation (Burr et al, 1989; Das, 2001; Hibbeln, 2002; Lawton, 2000; Ljor et al 2000; Mori et al 1999; Watkins et al 2000).

Seafood has an excellent nutritional value. It is defined by its high content of micro- and macronutrients. The contribution that seafood can make to the supply of micronutrients like selenium, iodine and vitamins A and D in the diet is well established. Over recent decades it has become clear that fatty fish and fish oil are a source of highly polyunsaturated fatty acids of the n-3 type (EPA and DHA). The anti-inflammatory effect of EPA and DHA in oily fish is believed to be protective against chronic nutritional disorders (e.g. cardiovascular disease), and effective in the treatment of other diseases that are mediated by an inflammatory process, (e.g. ulcerative colitis and rheumatoid arthritis). Recently, supplemental fish oil has been demonstrated to reduce bone turnover in rats and to ameliorate bone loss in elderly women. It has been suggested that these effects are mediated by an anti-inflammatory process. EPA and DHA may play a role in brain development and brain function and could have a positive regulatory role in metabolism, including improving insulin sensitivity.

There is increasing concern that the ratio of n-6:n-3 fatty acids in the diet in parts of Europe is too high, in the region of 1:12 where a desirable level is estimated to be nearer to 1:6. By consuming more fish, this imbalance could be rectified. However, it is not clear what the magnitude of these beneficial effects is for the consumer compared to consuming alpha linolenic acid (ALA) from plant sources. Although humans are able to synthesize the highly polyunsaturated n-3 fatty acids that are found in fish from ALA, it is not known whether this synthesis can produce optimal amounts of these highly poly unsaturated fatty acids *in vivo*. It is also possible that the synthesized quantities of these fatty acids are dependent on the composition of the diet, particularly on its content of linoleic acid and on genetic polymorphisms.

It is unclear what aspect of fish consumption confers protection on the large intestine. Fish mostly consists of protein and it is well recognized that fish protein has a high biological value as it provides relatively large amounts of indispensable amino acids. So far, little attention has been given to specific amino acid sequences in fish protein, which could provide bioactivity beyond traditional nutritional value, like anti mutagenic activity and antioxidant activity. Moreover, in addition to its high content of essential amino acids, fish proteins are known to be highly digestible in the gastrointestinal tract. A high ileal digestibility of proteins will result in a lower loss of essential amino acids into the colon and also in less protein fermentation in this organ by the intestinal flora. Less protein fermentation in the colon will result in lower formation of toxic compounds, e.g. ammonia, indoles and hydrogen disulfide. This is most likely beneficial for the colonic epithelium and may help to decrease inflammatory conditions, such as inflammatory bowel disease. Ileal digestibility of fish proteins and its consequences for the metabolism of the intestinal flora have not been investigated before

Other components from fish with potential bioactivity

Figure 2 shows a number of compounds from fish or shell fish. A number of these compounds could also exert beneficial bioactive properties, but in general the scientific substantiation of a positive effect on health is by far incomplete and thus health claims are not yet possible.

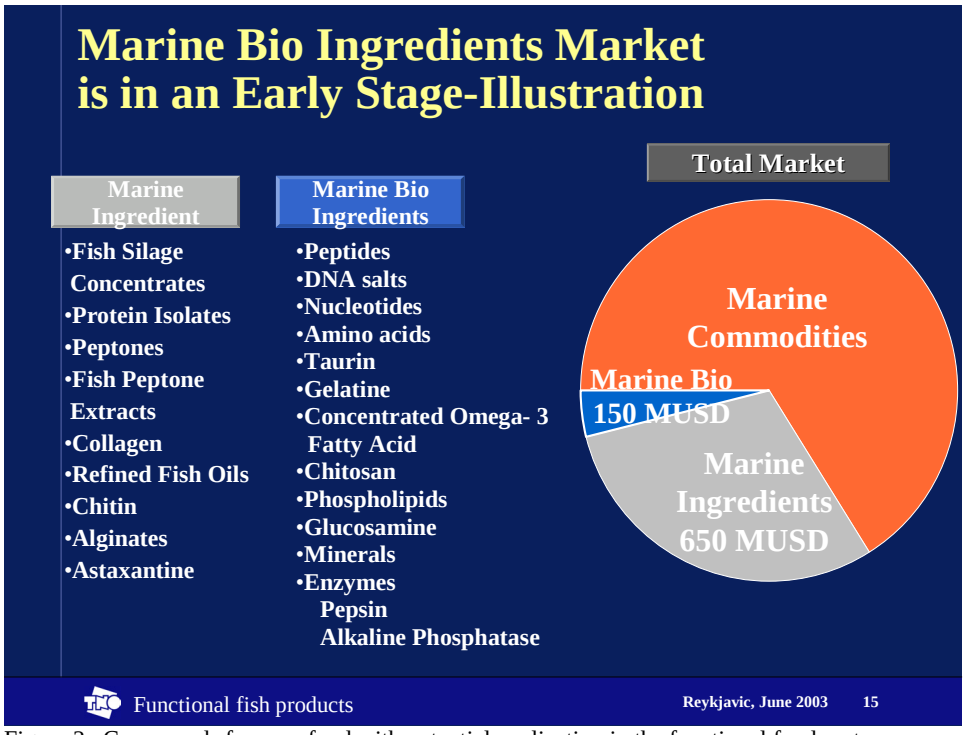

Figure 2. Compounds from seafood with potential application in the functional food sector

Taken from Dr. Tage Affertsholt, 3ABC Business consulting, Aarhus, Denmark

Taking into account the millions of tons of waste from fish, it is a great challenge for the fish-processing industry to valorize the waste streams. The growing market of innovative health foods creates here interesting opportunities.

Conclusions

The following conclusions can be drawn:

1. Fish is a product with an excellent traditional nutritional value, taking into account its content of a large variety of primary nutrients.
2. From the perspective of functional food development beneficial effects of n-3 fatty acids from fish are well documented, particularly in relation to reducing the risk of cardio vascular disease.
3. N-3 fatty acids and fish protein may have additional beneficial effects in reducing the risk of chronic diseases, e.g. colon cancer, osteoporosis, metabolic syndrome, and inflammatory diseases. However, further research is needed to substantiate these beneficial effects.
4. Fish contains a large number of other compounds that could serve as a functional food ingredient, but the scientific evidence for health claims of any of these components is still weak.

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L 17. SUPPLEMENTATION OF THE DIET OF HAEMODIALYSIS PATIENTS WITH PORTUGUESE CANNED SARDINES AND EVALUATION OF ω3 FATTY ACID LEVEL IN ERYTHROCYTE PHOSPHOLIPIDS

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Abstract

Nowadays, it is well known that a diet rich in fish lipids is responsible for the prevention of several pathologies, in particular cardiovascular diseases due to the modification of risk factors through mechanisms related to lipid metabolism. Thus, the increase of the level of eicosapentaenoic acid (EPA-20:5ω3) and docosahexaenoic acid (DHA-22:6ω3) in phospholipids is one of the lipid changes responsible for this prevention. Haemodialysis patients have a high risk of cardiovascular disease and are a potential group of study to evaluate the beneficial effect of ω3 fatty acids supplementation. Having this in mind, a group of 23 haemodialysis patients and a control group with 10 healthy elements were submitted to a diet supplemented with Portuguese canned sardine (4.3 g of ω3 fatty acids; EPA 2.1 g and DHA 1.33 g) three times *per* week during 3 months. The fatty acid profile of phospholid erythrocytes was evaluated in the beginning and in the end of supplementation. Initially EPA was lower in haemodialysis patients (0.56 %) than in control group (1.33 %). After 3 month of supplementation the percentage of EPA increased significantly in both groups respectively 1.5 % and 2.5 % for haemodialysis and control group. The percentages of DHA and docosapentaenoic acid (DPA-22:5ω3) were similar before supplementation (1.79 %) in patients and (1.96 %) in control group but after supplementation an increase was registered for DPA in the control group (2.67 %). Nevertheless, the DPA/EPA and DHA/DPA ratios used to evaluate the desaturases and elongases enzyme activity were similar in both groups after supplementation suggesting an active system of these enzymes in control group and in haemodialysis patients.

The aim of the present work was to evaluate the impact of a modified diet enriched with ω3-fatty acids, on the erythrocyte phospholipids composition.

Materials and Methods

A group of 23 haemodialysis patients (with chronic haemodialysis for more than 6 months) and a control group with 10 healthy elements (without diabetes or dislipidemias) were submitted to a diet regimen where 100 g of canned sardine were consumed three times per week for 3 months. Individual blood sampling was carried out before and after the supplementation study. Lipid classes distribution was performed according to Christie and Urwin (1995) and fatty acid profile of erythrocyte phospholipids was analysed following the procedure described by Bandarra and others (1997).

Results and Discussion

The fatty acid profile and the fat content of canned sardines are shown in Table 1. It has to be stressed the high level of ω3 polyunsaturated fatty acids, particularly, EPA and DHA that represents around 80% of total ω3 fatty acids. The high level of monounsaturated fatty acid is due to oleic acid (18:1ω9), which is the main component of olive oil of the packing medium.

Table 1. Fat content and fatty acid profile of canned sardine.

Fat and fatty acids	Percentage (g/100 g edible part)
Σ Saturated	4.07
Σ Monounsaturated	5.92
Σ Polyunsaturated	5.46
Σ ω3	4.30
EPA	2.01
DHA	1.33
Total fat	17.0

The evaluation of the fatty acid profile of the phospholid erythrocytes was carried out in the beginning and after the supplementation period in the control group (fig. 1) and in haemodialysis patients (fig. 2). The first aspect to be stressed is the higher level of arachidonic acid (AA – 20:4ω6) and EPA in the phospholipid erythrocytes of

the control group than in the haemodialysis patients before supplementation. In fact, initially EPA was 0.56 % in haemodialysis patients and 1.33 % in the control group. Nevertheless, after 3 month of supplementation the percentage of EPA increased significantly in both groups respectively 1.5% and 2.5% for haemodialysis and control group. As it can be seen in figures 1 and 2 the consumption of canned sardine led to an increase of the level of DHA in the control group and a decrease in the patient group. A similar trend was recorded for docosapentaenoic acid (DPA-22:5 ω3). In the beginning of the experiment, the level of this fatty acid was 1.96% and 1.79 % in the control group and in the patient group, respectively. Nevertheless, the DPA/EPA and DHA/DPA ratios used to evaluate the desaturases and elongases enzymes activity were similar in both groups after supplementation suggesting an active system of these enzymes in control group and in haemodialysis patients. The results obtained suggest that the diet supplementation with canned Portuguese sardine induce a significant increase in EPA erythrocyte membrane in both groups studied. Nevertheless, only a partial correction of EPA level was achieved in haemodialysis patients, suggesting a permanent deficient absorption/incorporation of EPA in this group, probably responsible for the initial very low level of this ω3 fatty acid.

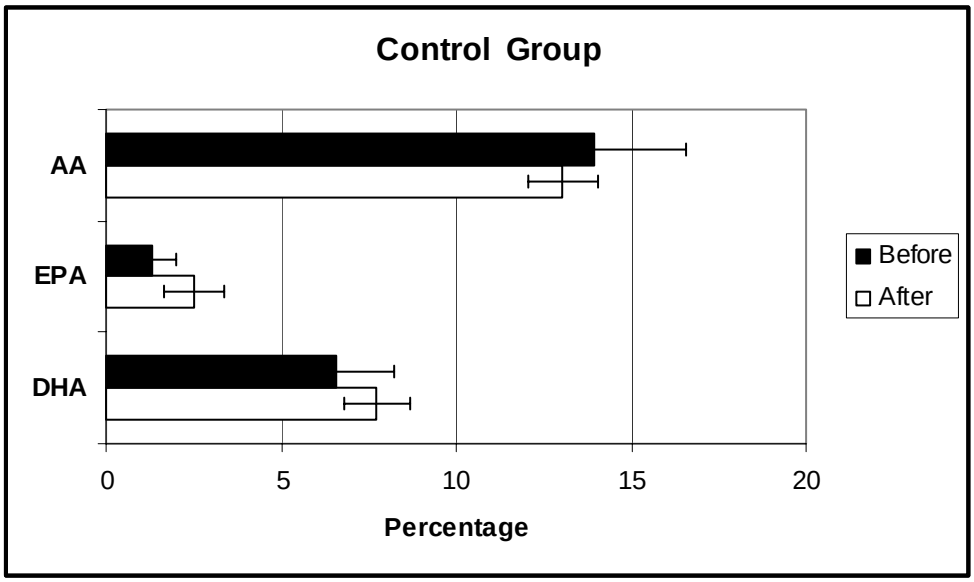

Figure 1 –Incorporation of AA, EPA and DHA in the erythrocyte phospholipids during the experiment in the control group.

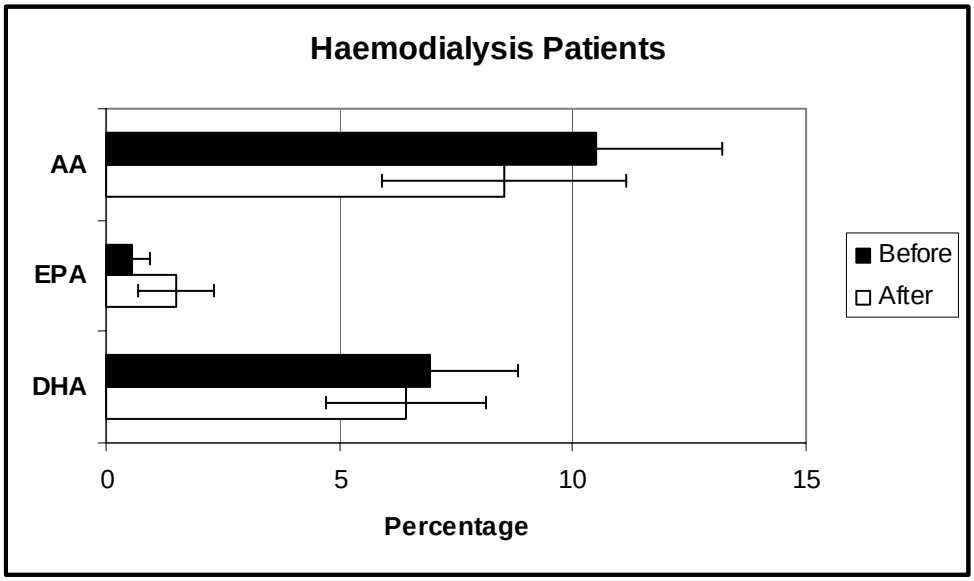

Figure 2 – Incorporation of AA, EPA and DHA in the erythrocyte phospholipids during the experiment in the haemodialysis patients.

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L 18. AVAILABILITY OF QUERCETIN AS FUNCTIONAL INGREDIENT IN FISH GELS

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Abstract

Quercetin, a natural antioxidant flavonol, was used as a functional ingredient in the preparation of surimi gels in order to study its antioxidant effects from technological and nutritional points of view.

Gels were made from surimi containing either 0.105 % quercetin, fish oil (group ω3), or fish oil additionally supplemented with 0.105 % quercetin (group ω3-Q). Antioxidant capacity, measured by DPPH•, was reduced when quercetin was added to the surimi to made the gel, suggesting a modification due to the technological process The addition of omega-3 fatty acids together with quercetin did not hinder its antiradical efficiency. Lipid stability was not enhanced due to quercetin since commercial fish oil already contains stabilizers. The addition of quercetin did not alter rheological properties nor microstructure but produced a large increase in yellowness (b*).

The gels were given to male Wistar rats for four weeks. Food intake and body weight gain were similar in the three groups, indicating good tolerance to this food. Plasma lipid profile of ω3 and ω3-Q fed rats was not significantly different from the control group in the experimental conditions . No structural modifications were observed in liver due to the diet. Total plasma antioxidant capacity, measured by the FRAP assay, was significantly increased only in the group containing quercitin. These results suggest that quercetin, added as a food ingredient in fish gel, was available in vivo and biological effects derived from its intake could add to the healthy effects of ω3.

Introduction

Quercetin is a common plant flavonol present in many fruits and vegetables (onions, wine, black tea, apples). Flavonoids have been widely investigated in recent years because of their potential antioxidant activity which improves the stability of lipid-containing foods (Chen and others, 1999; Bekhit and others, 2003) and to their possible beneficial effects on human health (Beatty and others, 2000; Dillard and Bruce German, 2000, Ramarathnam and others, 1995; Rice-Evans, 1999). The current market opportunities for nutrition/health foods are expected to increase in number and in consumer interest. The development of functional seafood products containing antioxidants would give added value to fish products that are, in themselves functional food products.

Even though flavonoids are generally regarded as good antioxidants, an assumption predominantly based on in vitro studies, however is little known about their actual in vivo capacity to function as antioxidants. Quercetin exhibited a stronger antioxidant activity than some synthetic antioxidants such as BHA and BHT to stabilize marine oils (Wanasundara and Shahidi, 1998).

The hypothesis is that quercetin adds a new functional component to the fish restructured product and at the same time helps retaining the health beneficial components intrinsically present in seafood muscle.

Materials and Methods

Frozen surimi grade A from Southern Blue Whiting (*Micromesistius australius*) off Chile with a moisture content of 74.96 % was used to gel preparation as described in Pérez-Mateos and others (2002). 1.5 % salt, 5 % starch, 2 % egg white, 0.105 % quercetin (Antrancine Q® from Altaquimica, Barcelona, Spain) and 8.3 % fish oil (ROPUFA™ '30' n-3 Food Oil, Roche Lipid Technologies, Derbyshire, DE) or 8.3 % sunflower oil. The commercial marine oils containing >30 % of omega-3 fatty acids already contains antioxidants to retard lipid oxidation and to decrease development of off-flavors.

The resulting batters were stuffed into cases (Krehalon Soplaril, Barcelona, Spain) of 85 mm thickness and 10 cm diameter (Walsorder K plus, Julio Criado Gómez, S.A., Madrid) and subjected to heat treatment. All the samples were cooled immediately and stored in a cold room at 4 °C overnight. The physico-chemical analyses were determined next day and the rest of gels were frozen and stored at -20 °C and they were thawed when necessary in order to feed rats along the experiment. The gels (containing either sunflower oil: group control; fish oil supplemented with omega-3 fatty acids: group ω3; and the same formulation additionally supplemented with 0.105 % quercetin: group ω3-Q) were given to three groups of ten male Wistar rats each for four weeks.

Rheological analysis, color, water holding capacity and microstructure of gels were determined. Also lipid oxidation was determined by changes in fatty acid composition and TBA index. Antiradical capacity was estimated according to the DPPH• procedure reported by Brand-Williams and others (1995), which was slightly

modified by Sánchez-Moreno and others (1998). Antioxidant capacity in plasma were determined followed FRAP analysis (Benzie and Strain, 1996). Plasma lipid profiles were also recorded.

Results and Discussion

Antioxidant capacity of quercetin as pure compound, measured by % remaining DPPH•, was significantly decreased when included in fish gels, by partial loss of activity after technological process. The addition of omega-3 fatty acids together with quercetin did not hinder its antiradical efficiency. Lipid stability was not enhanced due to quercetin since commercial fish oil already contains stabilizers. However it is well know the protective effect on lipid oxidation by quercetin (Ramanathan and Dass, 1992; Wanasundara and Shahidi, 1998). The addition of quercetin did not alter rheological properties nor microstructure but produced a large increase in yellowness (b*). Another authors (Chen and others,1999) also reported than pork patties with quercetin had yellow color.

Food intake and body weight gains of rats were similar in all the groups, indicating good tolerance to this food, no showing any nutritional deficient. Plasma lipid profile of ω3 and ω3-Q fed rats was not significantly different from the control group. No structural modifications were observed in liver due to the diet. Total plasma antioxidant capacity, measured by the FRAP assay, was significantly increased only in the group containing quercitin. Manach and others (1995) studies reported that quercetin was recovered in rat plasma as conjugated metabolites, and plasma had a yellow color with a peak absorbance at 375 nm due to quercetin.

These results suggest that quercetin, added as a food ingredient in fish gel, was available in vivo and biological effects derived from its intake could add to the healthy effect.

Acknowledgements

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L 19. MUSCLE FOODS WITH ALTERED COMPOSITION : RAINBOW TROUT (*ONCORHYNCHUS MYKISS*)

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Abstract

The research on muscle food with altered composition has again been gaining momentum lately because of the general public's interest to stay healthy.

Pioneering work on foods with altered composition were done by Canadian companies who boosted levels of omega 3 fatty acids in eggs; produced whole grains; oat, wheat and barley with enhanced amounts of soluble and insoluble dietary fiber; and modifed fatty acid vegetable oils, legumes and fruit products(Yalpani, 2002).

The research on muscle food with altered composition did not stay behind. Early work on fish was made on the enhancement of fish pigmentation in farmed raised fish. Fish feeds were augmented with carotenoids colorants. Work that succeeded thereafter, included boosting anti-oxidants in pork and poultry.(Decker, Faustman and Lopez-Bote, 2000)

Our research focused on three(3) bioactive compounds; Fish oils, carotenoids and CLA(Conjugated linoleic acid). Our intent is to produce cultured fish with higher levels of these bioactive substances in the fish muscle than normal cultured fish. These bioactive substances are introduced to the fish, through its feed. Rainbow trout(*Oncorhynchus mykiss*) was used as the target fish.

The results of our work suggested that Diets with augmented carotenoid pigmentation are significantly better than Diet(Control) without added pigmentation. Furthermore, CLA is not being synthesized, not unless incorporated in the feed diet. This is very interesting as there are very little publications on fish being or not being able to synthesize CLA from the diets. Results also suggest that fish oils(EPA and DHA levels) are not being synthesized, not unless incorporated in the feed diet. Thus, the results now suggest the importance of these bioactive compounds; carotenoids, CLA, EPA and DHA, in the fish diets, if these are to have maximum levels in the fish muscle.

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P 12. TAILORED, FUNCTIONAL, FISH CANNED PRODUCTS

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Abstract

A new concept and technology for the production of different functional fish canned products, which are the source of the very important in the diet n-3 PUFA, has been developed. Technological and scientific rules have been described of design of the raw material composition of the cans as well as the final nutritive content of the final product including: proteins, fat, mineral substances and some vitamins, together with the assured quantity of n-3 PUFA above 1.5 g/100 g and a controlled energy value of the canned products.

The production of the cans according to the new technology allows the tailoring of the products, ensuring that those have specially designed nutritive values adjusted to the defined needs of consumer segments, i.e.: children, elderly, people with diagnosed health problems, obese people, military, ship staff and crews as well as other closed segments.

The possibility do differentiate the nutritive value of the canned fish produced from the same raw material (the Atlantic mackerel *Scomber scombrus* with a 25.5% fat content in meat) utilizing the developed technology and procedures are presented (table 1) showing the two types of fish-vegetable canned products: type 1 – low energy value, type 2 – high energy value.

An additional positive side of the developed technology is the possibility to produce directly from raw fish meat. Because of that the amount of waste products is minimized and the loss of nutritive substances is also much lower, additionally, the process is simplified and much more energy efficient with increased final production yield.

The developed technology seems to present a promising direction in the optimization of fish raw material utilization for the production of tailored and functional seafood products.

Table 1. The differentiation of nutritive values in tailored fish-vegetable canned products, type I – low energy value, type II – high energy value, from Atlantic mackerel, fat content of 25.5%.

NUTRIENTS	TYPE I	TYPE II
ENERGY VALUE, KCAL/100 G	174,0	236,0
PROTEIN, G/100 G	9,9	18,8
LIPIDS, TOTAL, G/ 100 G	14,6	22,9
N-3 PUFA, G/100 G	3,5	4,6
CARBOHYDRATES, TOTAL, G/100 G	3,2	6,7
DIETIARY FIBRE, G/100 G	1,6	3,1
SODIUM, NA, MG/100 G	361,0	265,0
POTASSIUM, K, MG/100 G	333,0	450,0
CALCIUM, CA, MG/100 G	39,0	42,0
MAGNESIUM, MG, MG/100 G	22,0	43,0
PHOSPHORUS, P, MG/100 G	151,0	196,0
VITAMIN A, MG/100 G	114,0	56,0
VITAMIN E, MG/100 G	0,9	5,2

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P 13. DEVELOPMENT OF A NOVEL SURIMI-BASED PRODUCT

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Abstract

Surimi, a Japanese term, is extremely functional due to the unique gelling properties of the fish myofibrillar proteins. Surimi is a versatile ingredient that can be shaped, coloured and flavoured to produce a range of exciting novel products. The purpose of this work was to develop a range of surimi-based seafood analogs or products and determine their shelf-life. Four different glazes were used to produce four different flavoured products; Balti (B), Lemon and Pepper (LP), Italian (I) and Rosemary and Garlic (RG) surimi-based seafood products were developed and the shelf-life (4°C x 12 days) was assessed. Hardness of all four surimi products significantly ($p<0.01$) increased over the 12-day assessment period, while cohesiveness was not significantly affected. RG flavoured product had higher ($p<0.05$) hardness values in comparison to the other products. TBARS values for raw and cooked products were below 0.8mg MDA/kg. RG flavoured product had lower ($p<0.05$) microbial count during storage. pH did not significantly change over time. Organoleptic analysis showed that B, LP and RG scored greater than 60% for overall acceptability, while I had greater than 50% for acceptability. This study revealed that I flavoured products scored lowest in terms of overall preference, while there was no significant difference between B, LP and RG.

Introduction

One of the seafood industry's greatest success stories is the development of surimi-based seafood products. Surimi seafood is a unique new, healthy and nutritionally satisfying food that can be moulded, shaped, flavoured and coloured, and is also versatile, convenient and safe (Lauro, 2000). Products are prepared by extruding the surimi paste into various shapes. Surimi is widely used as an intermediate product for a variety of fabricated seafoods such as kamaboko, imitation shrimp, surimi sticks, chikuwa, fish cakes and fish nuggets (Lee, 1992; Venugopal and Shahidi, 1995). Apart from imitation products, surimi based products has received very little attention in terms of research and development, particularly in terms of shelf-life evaluation. Thus, the objective of this study was to develop a novel surimi-based seafood product and determine its shelf-life.

Materials and Methods

Whiting surimi was produced using traditional methods. Ingredients used in the production of surimi analogs (10% surimi, 73% minced whiting, 14% water, 2% coarse rusk and 1% salt) were mixed in a bowl chopper. The mixture was then shaped using moulds and flavoured by tossing the final shaped product in a bag containing glaze. Four different glazes were used to produce four different flavoured products; balti (B), lemon and pepper (LP), Italian (I) and rosemary and garlic (RG). These flavoured analogs or products were then modified atmosphere packaged (MAP) with a gas mixture of 40% carbon dioxide and 60% nitrogen for a shelf life trial (0-4°C x 12 days). Textural properties were evaluated using the SMS Texture Profile Analyser. Lipid oxidation was assessed by measuring thiobarbituric acid reacting substances (TBARS) using the method of Ke *et al.* (1977) and pH was also assessed. Both mesophillic and psychrotrophic counts were determined by spread plating onto plate count agar. The analogs were cooked on a pre-heated grill at medium heat for a total of 12 min (6 min each side). An in house panel (untrained but were experienced in sensory evaluation of various other food products) consisting of 12 people performed sensory analysis.

Results and Discussion

Hardness of all four flavoured surimi-based products significantly ($p<0.01$) increased over the duration of the study. RG flavoured product had significantly ($p<0.05$) highest hardness, while, hardness of B, I and LP were not significantly different. No significant differences were recorded between cohesiveness values of the four different flavoured products. TBARS values were significantly ($p<0.05$) affected by time. TBARS values initially decreased, however continued storage after day 8 resulted in an increase in TBARS values. TBARS values for LP flavoured raw products were higher ($p<0.01$) than values obtained for products flavoured with B and I, but not significantly different to RG. Moreover, RG gave a non-significant difference when compared against I and B over the same storage time.

In all products psychrotrophic counts ranged from 10^4-10^6 cfu/g while mesophilic counts for all products ranged from 10^4-10^5 cfu/g. RG flavoured product had lowest ($p<0.05$) psychrotrophic and mesophilic counts. This may be due to antimicrobial activity of RG. Numerous studies have reported the antioxidant and antimicrobial properties of rosemary and garlic (Aruoma *et al*., 1997; Del Campo *et al*., 2000). There was no significant

difference in pH between B and I, which ranged from 6.1 to 6.4, however, the pH of LP was lower ($p<0.05$) than B and I in the range of 5.7 to 6.1. The lowest ($p<0.05$) pH was found in RG flavoured product yielding pH values in the range of 5.2 to 5.8.

The sensory attribute of texture in B, I and RG decreased ($p<0.05$) from day 0 to day 6, while texture for LP increased ($p<0.05$) from day 0 to day 6. B scored highest for texture on day 0, but LP scored highest for texture on day 6. There was no significant difference in chewiness and flavour scores between the four products. Consumer acceptability of I and RG decreased slightly over time, whereas acceptability of LP increased slightly over time, but these were not significant. RG scored highest for preference on day 0, B scored highest on day 2 and LP scored highest on day 6. Preference scores for I were lowest on day 2 and 6. Overall visual assessment showed that B and LP scored higher ($p<0.05$) than I and RG for colour quality and acceptability during 12 days of storage in a refrigerated retail display cabinet.

Conclusion

With the exception of imitation shellfish products, surimi based foods have received very little attention in terms of research and development, or shelf life quality. In this study four different flavoured novel surimi-based products suitable for the chilled market was developed and its shelf-life assessed. Organoleptic, textural, colour, microbial and lipid oxidation analysis suggested that the products retained a high quality when MAP packed and stored for 12 days under refrigerated (4°C) display. While, results showed that there was no significant difference in overall consumer preference for test products flavoured with B, LP and RG, products flavoured with I scored lowest.

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P 14. PROGRESS OF LIPID OXIDATION IN DIFFERENT FISH OIL ENRICHED MILK EMULSIONS SUPPLEMENTED WITH EDTA

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Abstract

A still increasing amount of evidence is supporting the proposed beneficial health effects of the (n-3) long chain polyunsaturated fatty acids (LC-PUFA). As marine oils are rich sources of the (n-3) LC-PUFA, considerable efforts have been made to incorporate marine oils into various food products. However, due to the high degree of unsaturation in their fatty acid profile, fish oil undergoes rapid oxidation, and the presence of trace-metal in these food systems enhance oxidation even further. In this study the oxidative deterioration of fish oil enriched milk emulsions based on either tuna or cod liver oil was investigated. The sensory off-flavour, concentration of lipid hydroperoxides and volatile secondary oxidation products, determined by dynamic headspace GC/MS, were monitored during cold storage. The effect of EDTA as metal chelator in the milk emulsions was also investigated.

The results showed that the emulsions containing cod liver oil with a slightly elevated initial peroxide value of 1.4 meq/g oxidised significantly faster than emulsions containing tuna oil with an initial peroxide value of 0.1 meq/g. Fishy off-flavour was detected in cod liver oil emulsions, while fishy off-flavour in the tuna oil emulsions was around the limit of detection throughout the storage period. Addition of 5 ppm EDTA reduced the development of undesirable volatile oxidation products significantly in the cod liver emulsions, although their fishy off-flavour were still distinct. As evident from both the original analytical data and from multivariate data analyses EDTA had no reducing effect on volatiles in tuna oil emulsions with the low initial peroxide value. In conclusion, it was possible to emulsify highly polyunsaturated fish oil into milk and obtain an oxidative stable emulsion, which did not develop fishy off-flavour during storage.

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P 15. STABILITY OF MICROENCAPSULATED N-3 COD LIVER OIL.

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Abstract

Epidemiological studies suggest that a diet high in polyunsaturated fatty acids such as those found in fish oil may have beneficial effects on human health. Polyunsaturated fats are, however, sensitive to oxidation. Microencapsulation has been used to protect oil from oxidation and convert it from liquid form to a powder, thus providing food manufacturers with new functional ingredients. However, oxidative stability of the microencapsulated fish oil needs to be good if it is to be used as a functional ingredient in foods.

In this project the oxidation and the stability of microencapsulated polyunsaturated lipids (fish oil) was investigated with respect to the level of protection supplied by different coating materials. The fish oil was microencapsulated using a Büchi B-191 Mini Spray Dryer. Coating materials were mixtures containing a protein (gelatin or caseinate) and different types of carbohydrates (lactose, sucrose or maltodextrin).

Methods for measuring oxidation of the fish oil powders without lipid extraction, using oxygen uptake, SPME-HS-GC/FID, and sensory analysis, were developed. With these methods we could determine the effect of each coating mixture on oxidative stability.

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P 16. DIETARY FIBER AS A FUNCTIONAL INGREDIENT IN RESTRUCTURED FISH

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Abstract

Wheat fibers (74 % cellulose and 24 % hemicellulose) of two sizes (av. length 80 µm, av. thickness 20 µm; av. length 250 µm, av. thickness 25 µm) were added in two proportions (3 % and 6 %) to different kinds of restructured fish products to study their effects on textural and chemical characteristics in the course of frozen storage. The object was twofold: to improve technological aspects; and to help increase the intake of fiber in population sectors (mainly children) who do not normally eat salads, fruits or other fiber sources.
Three kinds of restructured products were used in the experiment: surimi gel, minced fish and a restructured product made from chunks discarded in filleting.
For the moment, we only have results for the preliminary analyses:

- Water can be added to the restructured products in a proportion of 3-4/1 water/fiber without significant loss of water binding properties in surimi and mince derived products.
- The texture of surimi and mince is significantly altered by addition of the fiber, but such changes may be desirable.
- Restructured products made with trimmings from filleting are most cohesive with 3 % added fiber. With higher proportions of fiber, the appearance of the product is unacceptable.
- Restructured products have a more attractive appearance if made from white fish.
- The test panel did not detect any odd flavors.

We intend to examine the influence of fiber addition on frozen stability of restructured fish products in the coming months. We also plan *in vitro* studies of the influence of fiber intake on the antioxidant capacity of plasma.

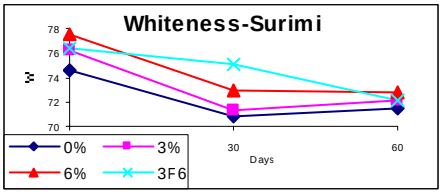
Materials

Gels. Surimi gels were made from Alaska pollack surimi (grade SSA) in a refrigerated vacuum homogenizer. Sodium chloride (2.5%) was added with sufficient iced water to give the required final gel moisture (74 %) in all lots. This dough was stuffed into 3.6 cm diameter Krhealon cases and heated for 50 min at 90ºC in a water bath.
Minced fish. The material was frozen hake fillets from *Merluccius capensis* caught 45 days before and stored at –25 ºC.
Restructured product from fragments. Fragments of about 3 x 3 cm were cut from fillets and divided into three lots. A portion of mince (about 20 %) was added to each lot. The fiber was first mixed with the mince and sufficient water to maintain the same moisture level as in the original muscle.
Fiber. Vitacel® wheat fiber was used: composition 74 % cellulose, 26 % hemicellulose and < 0.5 lignin. Average fiber lengths are: long fibre (Vitacel® - WF200) 250 µm and short fiber (Vitacel® - WF600) 80 µm

Results and Discussion

Appearance of samples. Gel samples made from surimi and from mince, both containing 3 and 6 % fibre, looked very much like fibre-free samples but were a little whiter. In the case of restructured product made from fillet fragments, when the fibre was directly mixed with the pieces, it clung among them and contributed significantly to post-freezing breakdown. When the fibre was first mixed with the mince and the appropriate amount of water and the resulting batter was mixed with the fragments, slices cut from the block were more cohesive and the fibre was less obvious.
Whiteness. Mince samples with fibre (Fig. 2) were clearly whiter than the control, and whiteness was proportional to the amount of fibre added. The proportionality was not so clear in gels made from surimi (Fig. 1), but fibre-free samples were less white than those containing fibre.
Instrumental texture analysis. Gel strength decreased in proportion to the amount of added fibre (Fig. 3). TPA analysis of mince showed the following: Hardness was lower with addition of fibre, although this was not dependant on the amount of fibre; Springiness and Cohesiveness also decreased when fibre was added. The cohesiveness index was proportional to the amount of fibre added. Loss of cohesiveness probably accounts for lower springiness and hardness (Figs 4, 5 and 6).
Water binding capacity (by centrifugation). Before freezing, water binding capacity was high and similar in control gel sample and sample with 3% fibre (Fig. 7), and was poorer in sample with 6% added fibre. After freezing, fibre-added samples lose more water in frozen storage.

In the case of mince (Fig. 8), fibre-added samples lost more water than the control; this loss of WBC became more apparent in the course of frozen storage up to 30 days.
Water holding capacity. The thaw drip is effectively captured by fibre, but it is only captured both in mince and fragments by long grain fibre (Fig. 9 & 11).
Cooking drip is captured by both short- and long-grain fibre, both in mince and fragments (Fig. 10 & 11).



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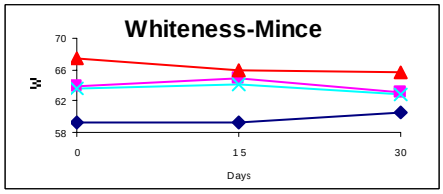

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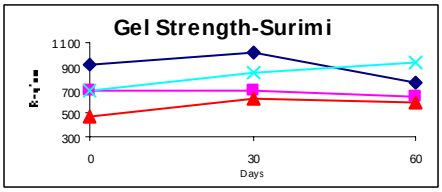

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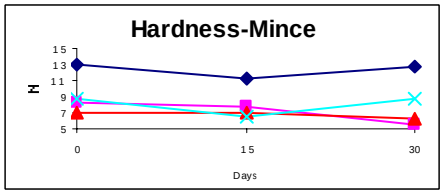

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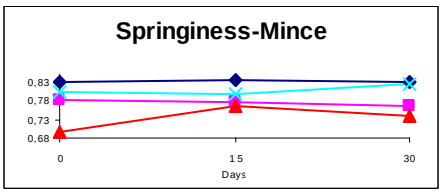

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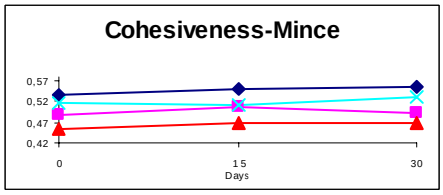

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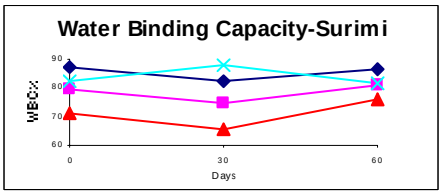

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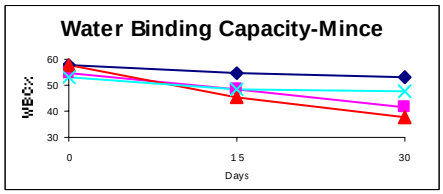

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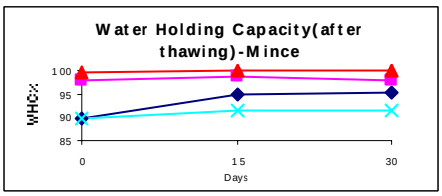

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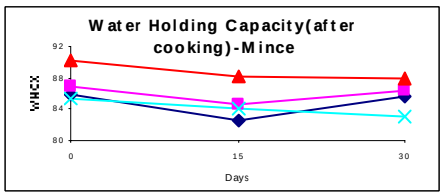

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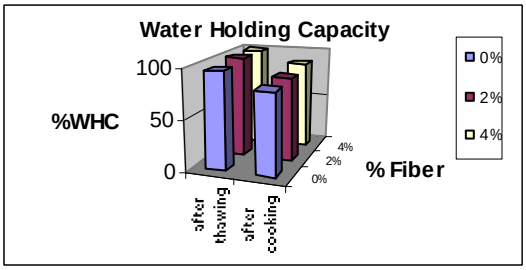

Figure 11

Conclusions

- Whiteness was improved when wheat fibre was included.
- Gels lost gel strength when wheat fibre was concluded.
- Minces lost springiness, hardness and cohesiveness.
- When intense force was applied, for instance centrifugation (and probably mastication), water was more readily released when fibre+water was added.
- When no force was applied, thawing drip and cooking drip were lower in samples containing long grain wheat fibre.

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P 17. UTILIZATION OF CRAB PROCESSING BY-PRODUCT IN THE DEVELOPMENT OF SEAFOOD RAVIOLI

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Abstract

Mechanical separation of crab processing by-product produces minced meat with good flavor which can be utilized in the development of value-added products. Fresh and frozen ravioli containing crab mince can provide a distinctive flair and utilize a resource that is typically discarded in the Northeast U.S. The objectives of this study were to develop a gourmet ravioli containing a significant amount of mechanically separated crab mince and evaluate its chemical, microbial, and sensory characteristics during fresh and frozen storage.

Mechanically separated Jonah crab meat (from crab processing discards) was mixed with chopped lobster, butter, bread crumbs, cheese, flour, and various spices in two separate formulations, and processed into ravioli using a commercial pasta machine. Triplicate batches of the stuffed pasta (120 ravioli/batch) were placed in plastic containers and stored at 4°C for two weeks, or at –20° C for three months. The pasta was periodically evaluated for total aerobic plate counts, TBARS, and total volatile base nitrogen (TVBN) and soluble protein at months. Both the fresh and 3-month frozen ravioli were evaluated for appearance, aroma, texture, flavor and overall acceptability by two 45 member consumer panels, using a 9-point hedonic scale.

The refrigerated ravioli had a shelf life of one week at 4°C, based on microbial levels. Fat oxidation was negligible (< 6 nmol TBA/g), however TVBN values reached 30 mg/100 g by week two. Frozen ravioli had a frozen shelf life of at least 3 months at

-20° C, based on chemical and sensory evaluation. Both the fresh and frozen ravioli were well liked by consumers, with highest scores received for flavor = 7.85, and overall acceptability = 7.74. The frozen ravioli is ready for the next stage of development however more research is needed to improve the microbial shelf life of the refrigerated crab mince ravioli.

Consumer perceptions to fish consumption

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K 3. ANTECEDENTS OF SEAFOOD CONSUMPTION BEHAVIORUR

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Abstract

This paper provides an overview of some recent findings on consumer attitudes and other important antecedents of seafood consumption and buying behavior. Taste, distaste (negative affect), nutritional value and freshness (quality) are suggested to be most important in forming consumers' attitudes and preferences towards buying and consuming seafood. Social norms, moral obligations and health involvement are probably more important motivational factors in explaining seafood consumption than for most other food products. Price/cost, convenience, knowledge and availability of top-fresh products are suggested to be important barriers towards seafood consumption for certain segments of consumers. This paper also discusses how and why consumption of seafood increases with age in several western countries.

Introduction

Seafood consumption varies greatly across countries. Top consuming countries are Japan, Iceland, Portugal, Norway and Spain. Even though differences exist between countries, the variation in consumption within the top consuming countries is high - from non-consumers to those who consume seafood on an almost daily basis (Olsen and Kristofferesen 1999). Like any complex human behavior, variation in seafood consumption will be influenced by many interrelating factors, such as properties of the food (quality and sensory attributes), characteristics of the individual (preferences, personality and knowledge), or characteristics of the environment (availability, situation, seasons and culture).

These phenomena give also the input for several theoretical approaches, models and methods for studying food consumption behavior (Shepherd 1989; Shepherd and Sparks 1994). A major distinction between studies of preferences and choice of food is ongoing between scientists with a strong focus on the product and product perceptions like sensory and quality evaluation (Rozin 1995), and scientists with the intention of explaining or predicting food consumption or buying behavior based on several antecedents in addition to product aspects (Shepherd 1989). Within the latter approach we find studies with different theoretical perspectives, included economics (Ritson and Hutchins 1995), psychology or sociology (Furst and others 1996), or general consumer behavior with a mixture of several theoretical approaches (Grunert 2002).

In this paper, I will discuss a number of antecedents of seafood consumption within a consumer and buying behavioral perspective. I will focus on some antecedents which I believe are of particular importance from both a research and industrial perspective, but with some biases towards studies on seafood as a family meal in some countries. In order to categorize and compare the research findings, I will primarily use the Theory of Planned Behavior introduced by Ajzen (1991) and further developed by others (e.g. Conner and Armitage 1998). This theory is the most used approach for studying human behavior including food consumption behavior, in recent years (Bredahl and Grunert 1997; Olsen 2001; Olsen 2003).

The theory suggests motivation or intention to consume as the primary driver of consumer behavior. Even though several models treat motivation or intention as a mediator between attitudinal variables, I will for patrimonial reasons use motivation to consume as synonymous with seafood consumption and buying frequency and a common dependent variable. The three focal antecedent factors to motivation and consumption are suggested to be attitude toward the behavior, social norms and perceived behavioral control. Because age seems to be a major factor in explaining variation in seafood consumption across countries (Brunsø 2003), age will be given some particular attention throughout this paper. The general model and factors to be discussed are presented in Figure 1.

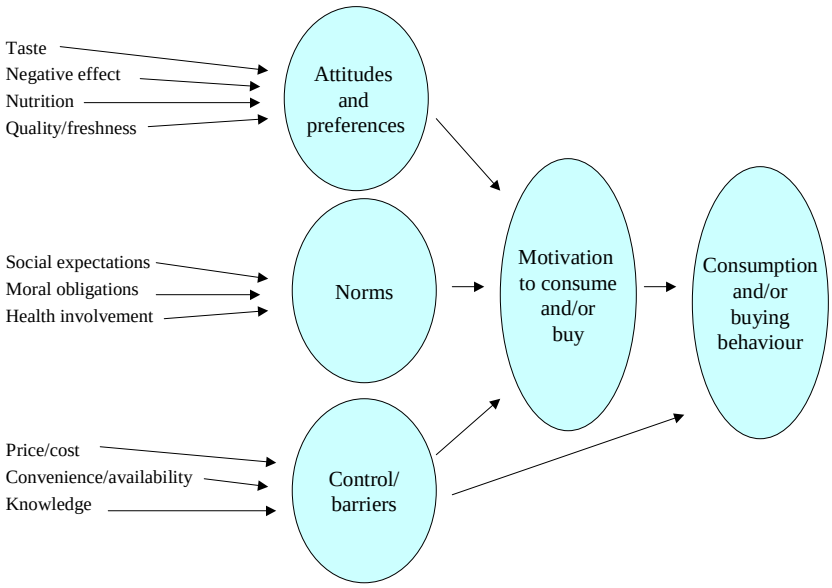

Figure 1 Important antecedents of seafood consumption and buying behavior

Attitudes and Preferences

Attitudes are suggested to be one of the main determinants of explaining food consumption behavior (Shepherd and Raats 1996), including seafood consumption behavior (Bredahl and Grunert 1997; Olsen 2001). Attitude is defined as a psychological tendency that is expressed by evaluating a particular entity (e.g. a food product) with some degree of favour-disfavour, liking-disliking, satisfaction-dissatisfaction or good-bad polarity (Eagly and Chaiken 1993). In this respect, it is theoretical arguments to include constructs like perceived quality of products, product preferences and acceptability, general likeability, satisfaction and affect as different facets of attitude or product evaluation (Olsen 1999). In order to investigate antecedents of general evaluation, it is common to evaluate different beliefs associated with the product. Taste, distaste (negative affect) and nutrition are suggested to be the most salient food attributes forming a general evaluation of food (Steptoe and others 1995), while quality and freshness in a more general sense are important for the evaluation of seafood (Olsen and Kristofferesen 1999; Olsen 1989; Grunert and others 1966).

Behind attitudes: Taste, negative affect and nutrition

Taste preferences seem to be the most important criteria when food is described either positively or negatively (Shepherd 1989). Taste or distaste is even more important for food choice among young consumers compared with the elderly (Berg and others 2000), which also seems to range nutrition and health as an important aspect of food selection (Roininen and others 1999). Several studies we are aware of prove that taste of seafood is among the most important factors in explaining general attitudes and motivation for buying and consuming seafood (Bredahl and Grunert 1997; Gempesaw and others 1995; Neuman and others 1995; Olsen 2001). On the other hand, we also find studies suggesting that taste and texture often play a minor role in determining consumers' associations with fish products (see Leek and others 2000 for a recent review).

Taste, however, is a matter of preferences. Some people like some kinds of seafood, and some don't. In general, seafood is less used and preferred compared than meat products in most countries we are aware of (Honkanen and Olsen 2001; Jonsson and Nilsson 1989; Letarte and others 1997). Most empirical studies indicate that elderly people in general eat fish or seafood more often than younger people do. This is confirmed in countries such as the USA (Nagaya & Capps, 1995), Japan (Olsen 1988), Taiwan (Li and others 2000), Norway (Myrland and others 2000; Olsen 1989) and Denmark (Grunert and others 1996). It is not unusual that elderly people consume seafood twice or three times as often as the younger generation does (Brunsø 2003). In a representative study among Norwegian teenagers, seafood seemed to be less preferred as a meal compared with chicken, meat products and the even more popular and modern dishes of pizza, taco and pasta-products (Honkanen and Olsen 2001). Seafood products received the lowest scores on liking and excitement, but highest on perceived nutritional values in a comparisons among about 25 meal solutions. Salmon was perceived as the most likable species, and fried fish was more preferable than boiled among Norwegian teenagers. However, preferences for seafood differ

among Norwegian teenagers. Honakanen, Olsen and Myrland (2003) found that ”fish lowers” covered about 30 % of the Norwegian marked for teenagers between the age of 12 and 19 years.

It has been suggested that the formation of positive (taste) and negative (distaste) attitudes toward food involves different psychological mechanisms (see Letarte and others 1997 for a recent review). Some attributes or beliefs contribute only negatively to the development of food attitudes. Unpleasant smell and bones have proven to be significant contributions in explaining less motivation or willingness to consume seafood across different countries (Bredahl and Grunert 1997; Leek and others 2000; Marshall 1993; Olsen 1989). In a study of Norwegian households, the negative affect of smell and bones explained 13 % of the variance in motivation to consume seafood (Olsen 2001). The impact is only negative because most consumers never buy seafood because of the bones and smell.

In several studies, nutritional aspects are considered to be the second most important product feature after sensory aspects and taste (Letarte and others 1997; Roininen and others 1999). Because almost everyone perceives seafood as some of the most nutritious food you can eat as a dinner (Brunsø 2003), it is hard to find a relationship between perceived nutritional value of seafood and seafood consumption (Bredahl and Grunert 1997; Olsen 1989). However, as I will later explain, nutrition is important in combination with involvement in health and healthy eating.

Quality and freshness

Quality of seafood is mostly determined by degree of freshness. It is a problem for the seafood industry that many consumers perceive frozen as the opposite of fresh. Consumer studies indicate that frozen is associated with “non-fresh”, “bad quality”, “tasteless”, “watery”, “boring” and other negative evaluations, which we do not find for fresh seafood (Olsen 1998). Extremely fresh seafood is associated with food for special occasions and a delicacy. A study by Olsen and Kristofferesen (1999) found large differences in attitudes towards fresh versus frozen seafood among Norwegian households. Even though, as expected, fresh was evaluated as being of superior quality compared with frozen, frozen is not only a matter of a technical property. Frozen seafood that consumers have frozen themselves (“home frozen”) seems to be evaluated as good as the fresh seafood they buy from seafood stores and better than fresh from supermarkets.

A reasonable explanation for this finding comes from trust towards the products and the problem of performing an appropriate evaluation of the frozen seafood in a shopping environment. Consumers feel more confident in their evaluation of fresh seafood because easily access to cues like appearance, texture and smell. Eight out of ten Norwegian adult consumers find it much more difficult to evaluate the quality of frozen seafood compared with fresh (Olsen and Kristoffersen 1999). The same results are found in USA, where Peavey, Work and Riley (1994) found that consumers generally felt that buying seafood was a risky venture that was even more pronounced when it came to frozen seafood. Frozen seafood was in this study associated with bad smell, mushy texture, bones, poor size selection and inferior taste.

As suggested by others in this session (Nielsen and Listov-Såbye 2003), however, the level and variation in technical quality of frozen seafood in many supermarkets may have contributed heavily to the “bad” image and expectations toward frozen seafood among many consumers. It is also a common attitude among consumers that fresh food in general is of better quality than frozen (with the exception of ice cream). Both meat and chicken products are exposed fresh in modern distribution, and fresh are increasingly promoted as branded quality items within the categories of meat, chicken, fruit and vegetables. All these aspects contribute to a general preference for fresh food. Our study (Olsen and Kristofferesen 1999) also confirmed that only 15% of the Norwegian consumers believe that frozen seafood is as nutritious as fresh, and less than 5% feel that the taste of frozen seafood is as tasty as fresh.

However, what consumers perceive as important and salient product beliefs differ across products and situations (Furst and others 1996; Marshall 1988). Leek, Maddock and Foxall (2000) suggest that frozen seafood has less association to bones and smell, which is an advancement compared to consumers’ perceptions of fresh seafood in the UK market. Kinnucan and others (1993) found that quality and flavor perceptions were important determinants for purchasing catfish; convenience was an important factor influencing the decision to purchase lobster; and nutritional value and health considerations were important determinants in the decision to purchase shrimp and cod in the US.

In order to signal quality and commitment towards consumers, the industries use different promotional cues in order to improve the perceived value, trust and loyalty towards the products. The most important cues are well promoted brand names. Warranty, high price, packaging attributes, information about origin, product content and production are also important cues in forming trust, commitment and loyalty towards food products, including seafood (Olsen 1995).

Norms, Morals and Health

Social norms and expectations

Social norms are often defined and measured as perceived social pressure or expectations from people in general (subjective norms) of form specific groups or individuals (Ajzen and Fishbein 1980). Social factors have been shown to be more important than genetic factors for the development of individual differences in food preferences (Rozin 1995). According to a review by Rolls (1988), family environment and peers are major determinants for children’s and teenagers’ food preferences. In several cultures, it is likely that families largely adapt their food choices to match individual family members’ food preferences (DeVault 1991). The foods that are

rejected by most family members are not likely to be served in the family (Koivistro and Sjödén 1996). Seafood is a matter of likes and dislikes, and many families feel ambivalence and conflicts when seafood is planned as a family meal. Bredahl and Grunert (1997) found that family expectations explained as much of the variance in both fresh and frozen seafood consumption as the attitude factor (taste- /- preferences) in Danish households. These results are similar to those found in Norwegian households by Olsen (2001). Children's dislike of seafood, or their strong preferences for hedonic consumption, is a barrier for seafood consumption in many modern households.

Moral obligations and health involvement

Investigators who have challenged Fishbein and Ajzen's claim that their norm construct is sufficient have shown that an individual perception of correctness or incorrectness of performing a behavior (internalized moral rules or obligations) needs to be distinguished from perceptions of others' ideas about what one should do or not do (social norms). Shepherd and Raats (1996) propose that the inclusion of moral obligation increases the predictive power of the theory of planned behavior when a person is involved in buying and preparing foods for other people, and perhaps particularly for children. Their propositions are based on studies testing people's attitudes towards food with artificial sweeteners and synthetic colorings, high-fat and high-sugar foods, and milk and food products produced by gene technology (Raats and others 1995). Olsen (2001) included a personal feeling of responsibility to take care of healthy eating and nutritional food within the family (moral obligation) as one of four antecedents for explaining variations in motivation for consuming seafood in Norway. Moral obligation proved to be the second most important predictor of motivation for consuming after negative feelings, and more important than attitude or preferences.

I believe that some of the explanations for why consumption of seafood increases with the size of the household (Myrland and others 2000) are explained by moral obligation. Single households and families without children do not feel obligated to serve healthy meals, provide alternatives to read meat and convenience foods, or demand varieties in their meals as compared with families with children. One reason why nutritional value is an important factor in explaining seafood buying behavior is nutritional value in combination with moral obligation and involvement in consuming healthy food. However, Foxall, Leek and Maddock (1998) proved that involvement in healthy eating is not always a main reason for purchasing fish when compared with taste or distaste. A healthy diet can be made up of an immense number of combinations of different foodstuffs, which may or may not include fish. This is also confirmed in my study (Olsen 2001) by a direct negative effect from moral obligation towards seafood consumption. Some people who are motivated to healthy eating choose chicken and other nutritional food as alternatives to seafood. Choosing these items leaves less room for using seafood as the one or two healthy dinners for the week.

In a study of the relationship between age and seafood consumption, Olsen (2003) proved a positive relationship exists between health involvement and the frequency of seafood consumption. Health involvement also had a highly positive influence on attitude towards eating seafood, and in that respect, an indirect effect on consumption in addition to the direct effect. This study suggested that one of the main explanations for the high correlations between age and seafood consumption is the mediating effect of health involvement. Elderly people are for different reasons more health conscious and involved in healthy eating than younger people (Roininen and others 1999). In other words, people with high moral obligation and who are involved in healthy eating are loyal seafood consumers. For this reason, families with children and elderly people are two important market segments for the seafood industry.

Perceived Behavioral Control and Barriers

Ajzen (1991) focused on perceived behavioral control as the person's beliefs as to how easy or difficult performance of the behavior was likely to be. The more resources and opportunities an individual thinks they possess, the fewer obstacles or impediments they anticipate, the greater their perceived control over the behavior should be. He suggests that control factors may either be internal to the person (e.g. skills, knowledge, lack of abilities, power of will, compulsion) or external to the person (e.g. time, opportunity, situation, dependence on others). Among the most important control factor that influences consumers' buying of seafood, I will discuss some barriers towards consumption with focus on aspects of price/cost, convenience/availability and knowledge.

Price and cost

In most countries, fish is perceived as expensive, and consumers indicate that the price level affects their intention to buy fish (Brunsø 2003). Leek, Maddock and Foxall (2000) did not find price and value for money as a significant factor for explaining variation in purchasing seafood in the UK, and some of the same results are found in Finland (Honkanen and others 1998). Household income and price were not perceived as a barrier for seafood consumption in a representative study of more than 4000 Norwegian women. Some respondents may not find price as a barrier to consumption because they do not purchase their seafood but, rather, rely on commercial or recreational fisherman in their household for their supplies. Another reason is that many households, that find fresh and high-quality seafood expensive buy cheaper alternatives of frozen and processed products, such as fish fingers of low-value raw materials.

Convenience and availability

Convenience proves to be a very important aspect of food choice (Steptoe and others 1995). Despite different definitions of convenience, most researchers within food consumption behavior relate convenience not only to an attribute of products, but to the capacity of consumers to employ particular resources, as well as simple time available (Gofton 1995). In a study by Furst and others (1996), time was mentioned as an important component of convenience, and people often spoke in terms of time as a commodity to be spent or saved.

Fish is regarded as very inconvenient because of the need to invest a large amount of time and effort, and devote special resources to various stages of the providing process (Gofton 1995). Fish is also inconvenient in some western households because it only goes with boiled potatoes and other ingredients, which take time to prepare. However, some fish products such as fish fingers or fish cakes are not regarded as inconvenient food (Marshall 1988; Olsen 1989). Convenience measured as "fish is readily available in shops", did not prove to be a significant factor (or item) in predicting fish purchasing among a random sample of about 300 UK consumers (Leek and others 2000). In an American study, convenience was an important factor influencing the decision to purchase lobster, but not for catfish, shrimp or codfish (Kinnucan and others 1993). Other studies of the US seafood market prove that convenience in some situations has an influence on seafood purchase (Gempesaw and others 1995). More than six out of ten Norwegian households would have bought more fresh seafood if it were more available (Olsen and Kristoffersen 1999). Elderly consumers perceive seafood as more convenient compared with younger consumers (Olsen 2003). This may be explained by more time allocated to buying and meal preparation and more knowledge accumulated over years by planning, providing and preparing seafood meals.

Knowledge

Knowledge of seafood is suggested to be an important factor in explaining choice of seafood (Brunsø 2003; Gempesaw and others 1995). Knowledge as an internal resource can be linked to several aspects, from evaluating the quality of the raw material in the shop to how to prepare and serve the final meal and its ingredients. However, monitoring lack of knowledge as a barrier for seafood consumption is a tricky issue. One problem is the fact that knowledge is highly correlated to frequency of use as a function of repeated experiences. Another issue is that knowledge may be important for some kinds of unprocessed seafood, for potential dishes and for the person preparing the meals for home consumptions. Moreover, knowledge may also be important concerning the perceived quality of and satisfaction with the final meal. It does not help to use the best raw materials if they are not prepared in the right way. Knowledge as a barrier for seafood consumption needs to be investigated with longitudinal design and under experimental conditions in the future.

Conclusions

As a conclusion, I suggest that seafood is less driven by hedonic, taste or positive sensory attributes compared with several other food products prepared for home meal solutions. However, extremely fresh products are accepted as high-value food in several consumer segments. Seafood is also associated with some negative attributes, such as bad smells and bones, which are possible to avoid by better quality management and product improvements. Seafood serves consumers' demand for healthy food and variety in their meals. It is driven by "housewives'" moral obligation to their partners and children. This is also one reason why elderly people and families with children consume more seafood than the younger generation and single households (Brunsø 2003; Myrland and others 2000) in several western countries.

I suggest that seafood is provided with more barriers towards consumption than a lot of other traditional food. This is not only because of conflicts in preferences in the households (teenagers often prefer other meals), but also because it is perceived as a high-risk and often high-cost product, difficult to evaluate and with limited availability of high and stable quality products with trust or commitment. The industry has a long way to go as concerns serving modern consumers with new, better and more convenient branded products. The competition from the meat and chicken industry in the area of product development and marketing is harder than ever. Understanding the market from the individual consumer's point of view is the challenge not only for the industry, but also for research on seafood quality and technology.

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L 20. BRINING OF COD: EFFECTS ON YIELD AND SENSORY PROPERTIES

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Abstract

Frozen and thawed fish products are often characterised by having lower eating quality than fresh ones. This is mainly explained by denaturation of proteins and leakage of moisture during both freezing, frozen storage and thawing. In order to prevent extensive denaturation of food during freezing and frozen storage, and thereby increasing the eating quality, additives like sucrose, glucose, phosphates, salt and antioxidants have been suggested included in different fish, meat and poultry products. For incorporation of additives to muscle tissue, different methods like tumbling, vacuum tumbling or injections have been used, as well as immersion or mixing minced muscle with additives

In this study, fresh and frozen / thawed cod fillets were treated with a brine consisting of salt, phosphates, sodium-ascorbate and glucose. The study focused on how brining of cod fillets affected yield and the sensory properties of the products. Sensory evaluations demonstrated that the intensity of the attributes cod smell, cod taste, glossiness, juiciness, flakiness and whiteness were higher in the brined products compared with non-brined. The effects of using different technological methods for incorporating additives are also evaluated.

Materials and Methods

8 products of cod having the characteristics given in Table 1 were evaluated both by the sensory panel and the consumers.

The brine consisted of salt (NaCl) polyphosphate, triphosphate, diphosphate, glucose and sodium-ascorbate.

The descriptive sensory analysis was performed with eight experienced and trained assessors at a sensory laboratory facilitating 10 panellists at a time. During training, the assessors established a vocabulary using samples spanning the anticipated variations and agreed on a list of 12 attributes. For intensity of the different attributes a continuously touch sensitive scale of 150 mm was used, anchored at the ends with “1” (low) and “10” (high). The sensory analyses were performed in two complete replicates. Samples of 50 g were sealed in aluminium foil and cooked in a water bath at 95ºC for 10 minutes. All the samples were served hot, being stored in isolated containers waiting to be served. The samples were blind-coded with three digit numbers, and served randomly.

Table 1: Characteristics of the 8 products used in this study

Product code	FrBFr	FrB	FrFr	Fr	FBFr	FB	FFr	F
Frozen/thawed before processing	X	X	X	X				
Fresh cod					X	X	X	X
Filleted and cutted	X	X	X	X	X	X	X	X
Brined	X	X			X	X		
Frozen/thawed before serving	X		X		X		X	

Results and Discussion

Table 2 shows the sensory description of the different products. In the table, the products are grouped as pairs according to non-brined/brined. The differences between the average scores given are largest for the pair FrFr/FrBFr where 7 of the 12 attributes have a numerical difference equal to or higher than 1. For the pair FFr/FBFr only three attributes have numerical difference equal to or higher than 1. These results indicate that brining has a stronger effect on products that was thawed before brining.

Table 2. The sensory description of the different products. *

Attribute \ product	FrFr	FrBFr	Fr	FrB	FFr	FBFr	F	FB
Cod smell	2,9	3,9	4,0	5,6	3,0	2,3	3,6	4,0
Old/stale smell	3,9	1,9	3,7	2,5	3,8	5,7	5,1	3,7
Glossiness	5,2	6,9	5,8	6,6	4,5	5,0	6,3	7,3
Whiteness	3,7	6,4	4,7	6,3	3,9	5,1	5,3	5,9
Flakiness	5,4	6,0	5,4	6,8	3,9	5,0	5,4	7,5
Cod taste	3,8	4,8	3,7	5,4	3,6	3,1	3,8	5,2
Salt taste	5,9	5,4	6,1	5,1	4,3	4,1	5,8	3,9
Old/stale taste	3,2	0,9	2,7	1,8	3,2	3,0	3,5	2,1
Wateriness	1,7	2,0	2,7	1,8	1,1	1,9	2,1	1,9
Fibrousness	5,7	5,5	7,0	6,1	5,4	4,7	5,7	5,8
Chewiness	6,0	5,5	7,4	5,3	4,8	4,9	5,9	5,2
Juiciness	4,7	6,0	5,6	6,5	5,3	5,4	5,8	6,0

*mean values from eight assessors

The results from the sensory description were analysed by using multivariate data-analyses. An unweighted principal component analysis (PCA) was performed on the sensory variables in order to achieve an overview of the main variations in the evaluations of the different products. The loadings of the first two PCs, representing 34 and 15% of the total variation, are presented in Fig. 1. The loading plot shows that the attributes related to odour (cod smell and old / stale smell) and taste (cod taste, old/stale taste and salt taste) are mainly described by PC1. The attributes related to texture (flakiness, fibrousness, chewiness and juiciness) are mainly described by PC2, while the attributes related to appearance (glossiness and whiteness) are described both by PC1 and PC2. Examination of the corresponding score plots, using sample grouping according to different treatments (frozen/thawed or fresh before processing; non-brined or brined; frozen/thawed or chilled after processing), show that whether the products were frozen or fresh prior to processing seems to affect taste and odour, and whether the products were frozen or chilled after processing seems to affect the attributes related to texture. On the other hand, brining affects both taste, odour and texture attributes.

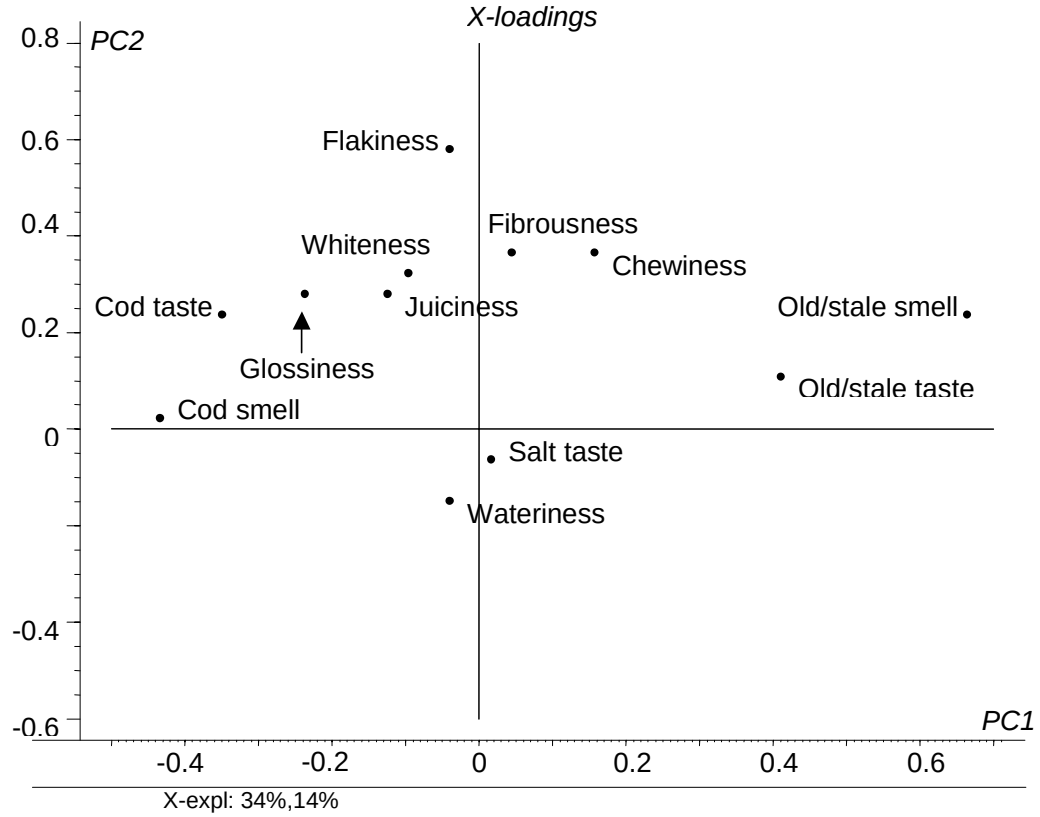

Figure 1. Loading plot for the first two principal components (PC1 and PC2) of the principal component analysis (PCA) model of the sensory evaluation. PC1 and PC2 explained 34 and 15% of the variations in the data set, respectively.

Conclusions

By treating cod fillets with brine consisting of salt, phosphates, sodium-ascorbate and glucose in a vacuum tumbler, the intensity of several sensory attributes were strongly affected.

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L 21. BRINING OF COD: ENHANCING CONSUMERS' PREFERENCE

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Abstract

Consumers do not have a common language to describe or explain their attitudes towards food. In food product development this is an obstacle to effectively translate i.e. likings and/or disliking into specific sensorial values. To do so one needs methodology that in an "indirect" way can explain why some products have a higher preference than others. By choosing the right tools to do so, one can find product related attributes that one can manipulate through the production process thereby enhancing the possibility of making products with increased consumer preference.

In this study 45 consumers voluntarily recruited among the staff at the Norwegian Institute of Fisheries and Aquaculture Ltd evaluated 8 products of cod. The participants were told that they were to evaluate pieces of filets from cod, with no reference to treatment or whether they were fresh or frozen. For taste the labels were ranging from "Very poor" (1) to "Very good" (7). The labels for texture ranged from "Very dry" (1) to "Very juicy" (7), while the labels for overall liking ranged from "Totally unacceptable" (1) to "Very acceptable" (7). The labels for salty taste were ranging from "Too low" (1) to "Too high" (5). The "Overall liking" was the last attribute to be evaluated.

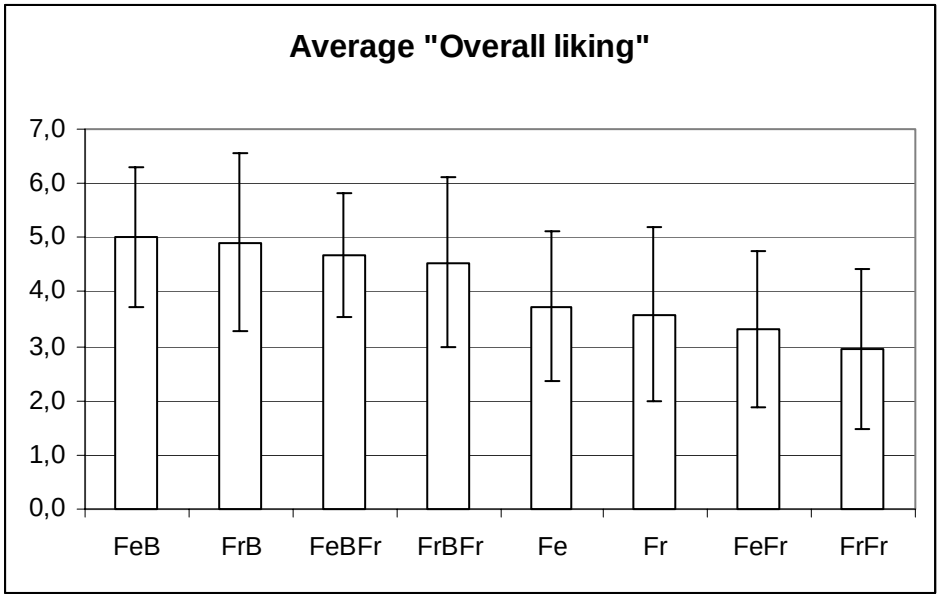

Figure 1: Results for the evaluation of "Overall liking" for the 8 products (N=45).
The products are defined as follows: Fe = Fresh Fr = frozen B= brined

As can be seen from figure 1, all the brined products (FeB, FrB, FeBFr and FrBFr), cluster on the left side of the fresh product (Fe). Especially remarkable is the shift in the double frozen product (FrFr) when it is brined after thawing and before refreezing (FrBFr). The average of "Overall liking" jumps from about 3 to 4,5. A pairwise t-test shows that this difference is statistically significant ($p>0,001$).

A descriptive sensory analysis was also performed using trained assessors. The intensity of 12 sensorial attributes was described for the 8 products. By combining the consumers' preference data and the sensorial data in

a multivariate analysis and simultaneously using Jack-knife techniques to omit attributes without statistical significance, it was found that the consumers overall liking (preference) of the 8 products could be explained by a limited number of sensorial attributes. This is demonstrated by looking at the relative positions of the sensory properties in figure 2 and the products in figure 3.

The conclusion was that brining the cod fillets in general heightened the consumer overall liking, and that the consumers preference could be explained by heightened intensities of cod taste, glossiness, juiciness, flakiness and whiteness, as well as lowered intensities of old / stale taste.

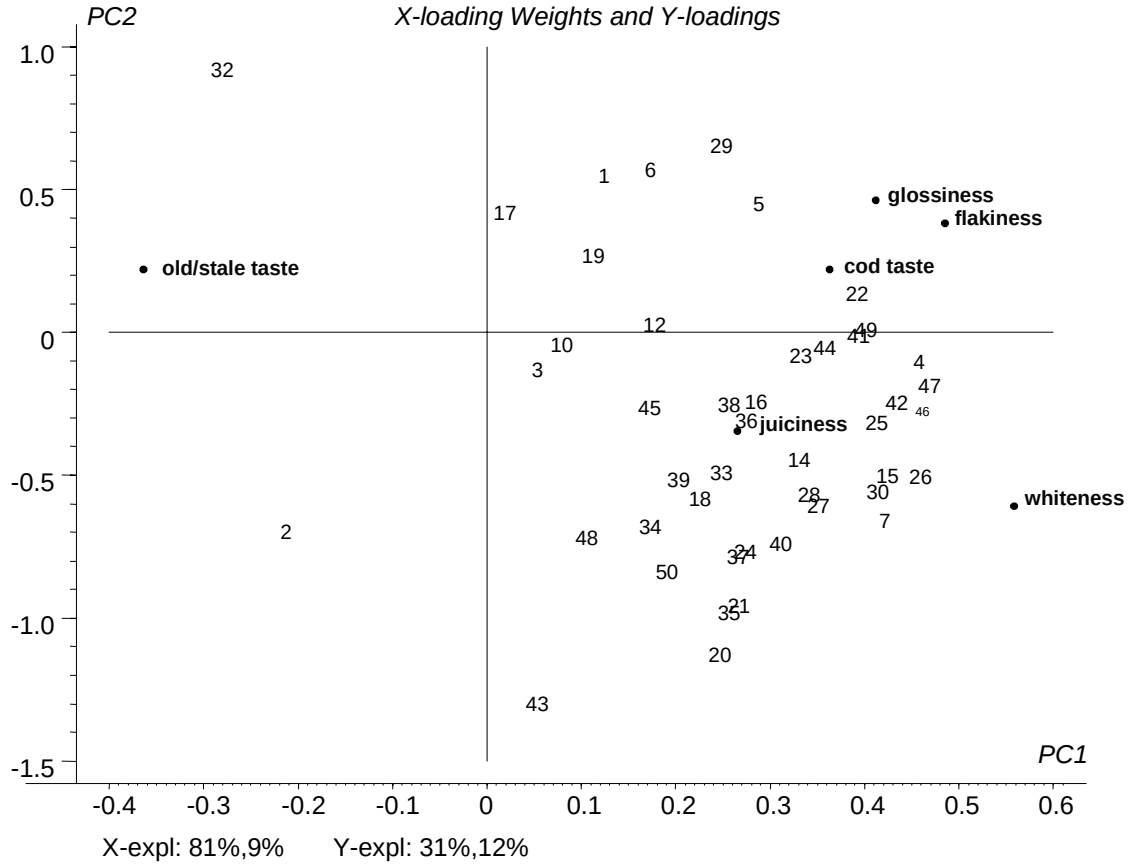

Figure 2: X-loading weights and Y-loadings for the two first principal component of a partial least squares regression (PLS2) model of the consumers "Overall liking" and the significant sensory attributes. The numbers identify the participating consumers.

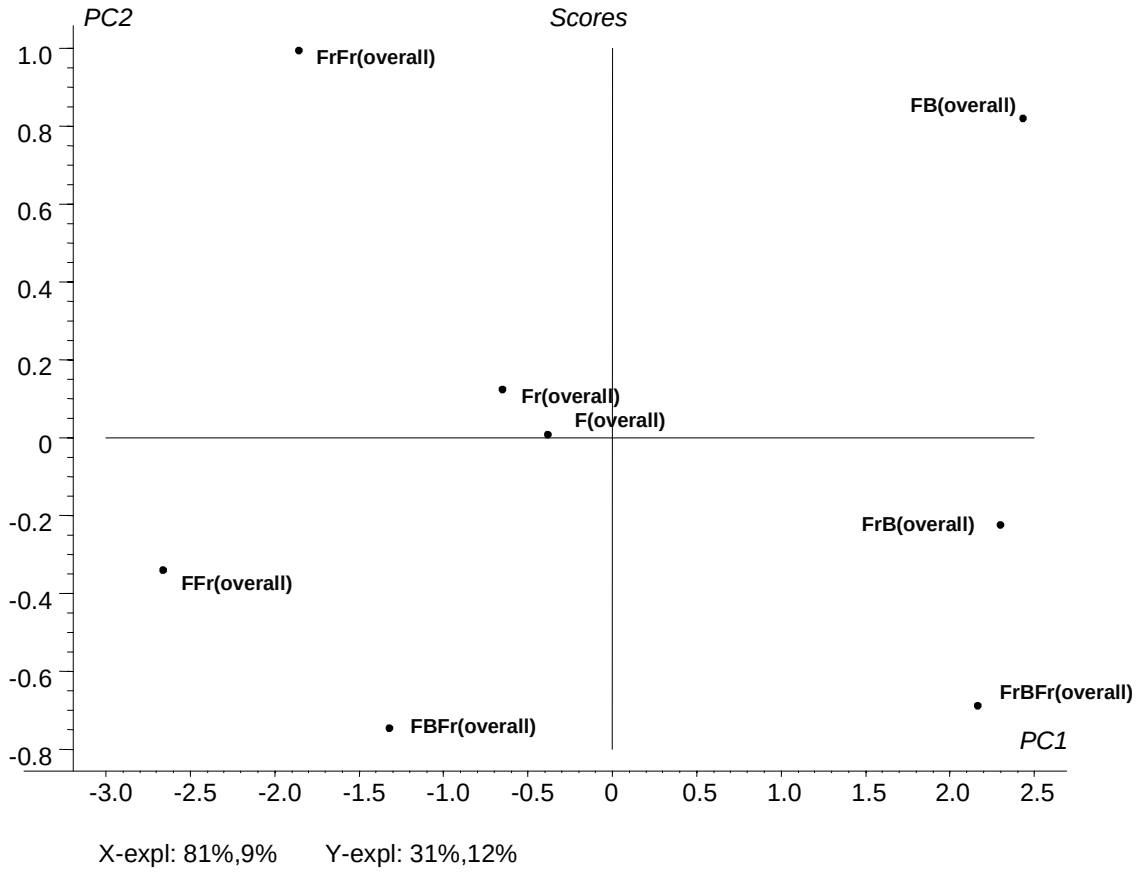

Figure 3: Score plot for the two first principal components a partial least squares regression (PLS2) model of the consumers “Overall liking” and the significant sensory attributes. (F=Fresh, Fr= Frozen B=Brined)

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L 22. CONSUMER PERCEPTION OF WILD AND FARMED COD UNDER DIFFERENT INFORMATION CONDITIONS: TWO IN-HOME CONSUMER STUDIES.

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Abstract

Fish farming is a booming industry. So far no results have been reported to show whether non-expert consumers perceive farmed cod to be different from the wild caught cod they are used to. Our first aim is to show whether non-expert consumers in their normal (in-home) consumption context perceive farmed cod to be different from wild cod. Secondly, the effect of (textual) product information on product perception is studied.

Two studies were performed using the Dutch TasteNet® in-home consumer panel. In the first study about 700 respondents from some 400 households judged wild raised cod and wild cod on two separate occasions. In a non-complete design the effect of information about the origin of the fish was tested (no information, “farmed”, or “wild”). In the second study (n=1400), effects were tested of production type (wild versus farmed cod), production type labelling (“wild” or “farmed”), price, time since catch, 'best before' date, quality control information, and the effect of knowledge about fish farming practices. In both studies expected and experienced qualities were measured, using analytic and hedonic attributes.

Results of the first study indicate that the respondents noticed little differences between wild and farmed cod. The average evaluative profile for *farmed* cod was almost identical to that of *wild* cod. Extra information (*labelling* “farmed” or “wild”), however, resulted in significant better scores for overall 'satisfaction' and 'attractiveness', and also for perceived 'smell' and 'colour'. No clear interaction of production type or labelling with experienced versus expected ratings was found.

These results will be tested and extended through the second study which will be finished in April 2003. These fresh results will test the hypothesis that consumers perceive no difference between farmed and wild cod as well as reveal the influence of more different information types on consumer product perception.

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L 23. CONSUMER SURVEY: COD FILLETS PACKAGED IN AIR AND MODIFIED ATMOSPHERE (MAP)

(not to be quoted without prior reference to the authors)

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Abstract

Preference mapping was used to compare consumer preference of fresh and thawed fish packed in air and modified air (MAP) and results of sensory evaluation by a trained sensory panel. The packed samples were stored for 2 and 10 days (0-1°C) before evaluation. Fresh cod packed in air and kept 2 days (1°C) received the highest sensory scores for e.g. freshness, juiciness and tenderness according to the sensory panel. The study showed that consumers found difference between different storage time of fish, 2 days and 10 days, prefering the more fresh fish. They most prefered the unfrozen MA-packed sample, the air packed thawed samples and the MA-packed thawed samples.

Introduction

The market of fresh chilled fillets has increased compared to the market of frozen fish in Europe. It has been stated that freshness of fish is very important to consumers. The aim of this study was to investigate the consumers preference of fresh versus sea-frozen (thawed) cod packaged in air and modified air (MAP). The results of the hedonic scoring of consumers were compared to the results of a trained sensory panel by preference mapping.

Materials and Methods

During 4 weeks period a sensory panel and a group of consumers evaluated 6 samples of cod. The samples are defined in table 1.

Table 1. Treatments and short names of the six different samples before sensory evaluation

Sample name	Description of samples	Packing method	Storage at 0-2°C before evaluation
A2d-fr	sea-frozen	air	2
A10d-fr	sea-frozen	air	10
M2d-fr	sea-frozen	MAP	2
M10d-fr	sea-frozen	MAP	10
A2d	fresh (newly caught fish)	air	2
M2d	fresh (newly caught fish)	MAP	2

Sensory evaluation: The sensory panel (11 trained individuals) evaluated each sample by the Torry freshness score sheet (Shewan et al. 1953) and Quantitative Descriptive Analysis (QDA) described by Stone and Sidel (1985) modified, as only odour, flavour and texture attributes were used. The samples were cooked at 95°C in a steam oven for 7 minutes before served to the panel.

Consumer test: In-home test was used for consumer testing. Regular fish consumers who eat fish once a week or more often (36 families, 110 individuals, age 11-77) were chosen to participate in the consumer test. The consumers received the samples ready for cooking at the same day. The consumers chose the recipe but each time they had to prepare and cook the fish in the same way without addition of spicy seasoning. All family members were asked to rate their overall liking on a 9-point scale, as well as their liking of flavour and texture.

Attitude survey: The person within each family responsible for food purchase filled in an attitude survey concerning fish and fish consumption.

Data analysis: Results from the sensory evaluation were treated in NCSS 2000 with analysis of variance. Duncan´s Multiple-Comparison Test was used to analyse difference between samples. Results from the consumer test were compared to results from sensory evaluation and interpreted with Internal preference mapping in Unscrambler 7.5 (CAMO A/S) using Principal Component Regression (PCR). The individual consumer scores were used as a X-matrix and the average sensory attribute scores were used as a Y-matrix as described by Helgesen et al. (1997).

Results and Discussion

Sensory evaluation: The results showed that the air packed samples stored for 2 days received the highest freshness (Torry) scores, but samples stored for 10 days the lowest scores for freshness. The unfrozen air packed sample received the highest freshness score. None of the samples had reached the end of shelf life, which was set at the Torry freshness score of 5.5 (Martinsdottir et al. 2001). The scores for sour odour, frozen storage odour and flavour and sour sweet flavour were highest for the air packed sample stored for 10 days. The texture scores of A2d were around 80, which was similar to earlier results (Martinsdottir and Magnusson 2001), but the M2d received lower scores for texture. The thawed groups received lower texture scores compared to the fresh groups and were evaluated drier and tougher.

Consumer test: The consumer scores of overall liking of the six fish products are presented in Table 2.

Table 2. Average scores of overall liking with standard deviation, median, highest and lowest values for overall liking on a 9-point scale. Different letters show statistical difference between samples

Sample	Average (StDev)	Lowest value	Median	Highest value
A10d-fr	5,3 (1,9)[a]	1	6	8
M10d-fr	5,8 (1,6)[b]	1	6	9
A2d	6,7 (1,5)[c]	2	7	9
M2d-fr	7,1 (1,2)[d]	2,5	7	9
M2d	7,2 (1,2) [d]	4	7	9
A2d-fr	7,6 (1,1) [d]	4	8	9

The consumers found difference between samples stored for 2 and 10 days and prefered samples stored for 2 days. Sample A10d-fr was least prefered and was the only product never receiving the maximum preference score (9). Samples stored 10 days were the only samples receiving the lowest possible score (1), and no consumer gave the two most prefered products, A2d-fr and M2d a score below 4.

Preference mapping: The consumer preference was more towards the thawed, air and modified air packed samples stored 2 days, or towards the modified air packed (unfrozen) sample but less the air packed sample stored 2 days (Fig. 1 a and b).

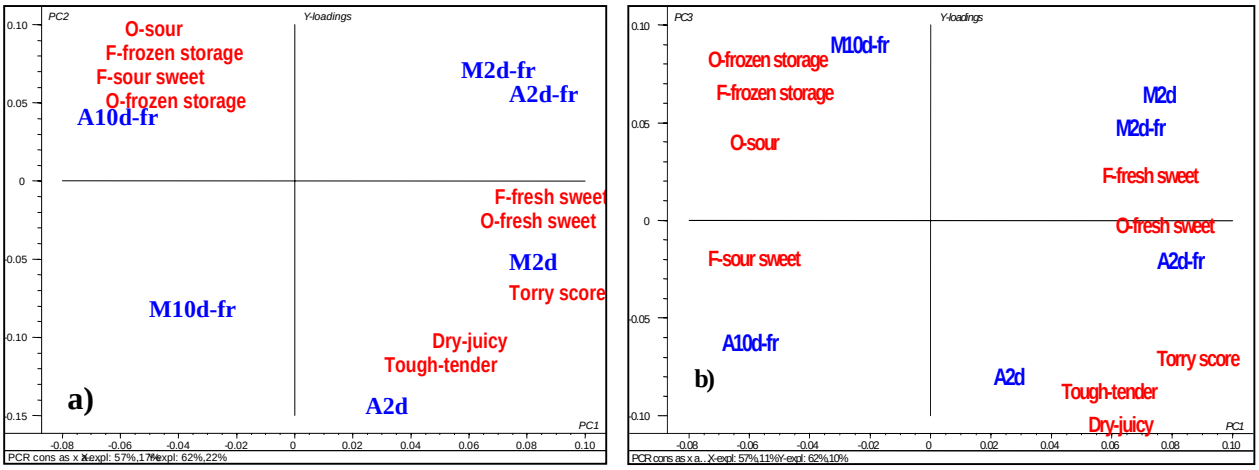

Figure 1. Internal preference components (consumers) and sensory attributes as evaluated by trained assessors. A = samples stored in air, M = samples stored in modified air packaging, 2d and 10d indicate the storage time in days, fr = samples frozen before air or modified air storage. O = odour, F = flavour. Y-Loadings of a) PC1 vs PC2 and b) PC1 vs. PC3.

However, the air packed sample (unfrozen) stored for 2 days received highest freshness scores according to the sensory panel. The air packed sample stored 2 days is related to juicy and tender in figure 1. Many of the consumers gave negative comments on the texture of this sample, usually describing it as too soft or "loose" texture. The MAP (unfrozen) sample was more prefered than the air packed (unfrozen) sample at storage day 2. The modified air may already have altered the texture and/or flavour of the fish flesh in these two days. The results from the sensory panel supported this, as the air packed (unfrozen) sample was significantly more tender compared to the sample packed in modified air (unfrozen).

The samples stored for 2 days were described as more fresh-sweet in odour and flavour compared to samples stored for 2 days, and received higher scores for freshness (Torry score). The samples stored for 2 days were also more tender and juicy compared to samples stored 10 days. The unfrozen samples appeared to be more juicy and tender compared to thawed samples. The preference mapping indicated that more frozen storage odour and flavour was of the modified air packed samples, which was in agreement with the comments of several consumers, describing the odour and/or flavour of the MAP samples as "unusual".

This study shows that consumers found difference between different storage time of fish (stored 2 and 10 days) packed in air and MA-packages, prefering the more fresh fish.

These results are a part of a project funded by the Icelandic Research Council (RANNÍS) and the Icelandic companies ÚA and ÍSAGA

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L 24. EUROPEAN CONSUMER PREFERENCE FOR SMOKED SALMON

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Abstract

As a part of the European project EUROSALMON (QLK1-2000-01575) which involves IceTec (Iceland-coordinator), Institute of Marine Research (Norway), Adriant (France) and IFREMER (France), a mapping of the preference of European consumer in selected countries within Europe i.e. Belgium, France, Germany, Italy and United Kingdom was undertaken as well as the mapping of sensorial, chemical and physical characteristics of smoked salmon sold on the European market.

For the European consumer study, 30 smoked salmons products were selected during a preliminary study; this was done in order to ensure that the range of characteristics of the different products sold in the European market were represented in the study. The products were ordered directly from the producers. Sensory evaluation was carried out with a trained sensory panel, and microbiological and chemical analyses such as: salt, phenol, TVB-N and TMA content were performed on each sample.

Results show that the product "smoked salmon" is a product which can be classified into different groups according to sensory properties and composition or aspect (phenol, salt, lipid level or colour). Evaluation of European consumer preferences has shown specific preferences according to product characteristics and five different classes of consumers have been identified. Difference in origin i.e. which country the consumers come from is not the main factor which explains consumer behaviour. It seems likely that a general European behaviour exists even though some regional preferences could also be observed.

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L 25. A CONSUMER VIEW OF FROZEN FISH

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Abstract

Several surveys have revealed a negative consumer attitude toward purchase of frozen fish products (Peavy et al. 1994; Anon. 1995; Nielsen et al. 1997). Frozen fish is associated with inferior taste and tough and dry texture and frozen fish is sometimes believed to have reduced nutritious value and to be bonier than fresh. Clearly new guidelines for production and especially storing of frozen fish in the chain from catch to consumer is needed. This have been given in several recent papers (Guldager et al. 1998; Bøknæs et al. 2001; Martinsdottir and Magnusson 2001; Bøknæs et al. 2002), where it is shown that it is possible to produce high quality frozen fish also in industrial scale when a few simple rules is respected.

In order to understand the consumers attitude toward frozen fish a three step investigation was planned: 1) consumer attitude to frozen fish using focus groups, 2) evaluation of the quality of frozen cod available on the retail market using objective methods, and finally 3) testing the consumers perception of frozen and thawed high quality cod.

METHODS

Focus group: Two focus group interviews was conducted after a method suggested by Morgan, 1998. The questions revolved around perception of quality, trust in retailers as well as information concerning food in general.

Testing of frozen fish bought at retailers:

20 packages of frozen fish (code 1–20) bought at 7 retailers were compared to 2 reference samples using chemical, physical and sensory analysis. The reference samples was vacuum packed cod fillets from the Baltic Sea stored at -30°C for 2 months (reference 1) and interleaved packed sea-frozen cod fillets from the Barents Sea stored at -30°C for 14 months (reference 2) as reported by Bøknæs et al. 2002. Thawing drip, water holding capacity, TVB-N, free formaldehyde was analysed among other things together with sensory profiling using a trained external panel.

In-home test: The effect of different treatments of cod was elucidated in an in-home test where 400 consumers were presented to different variation of frozen and thawed cod fillets positioning them to evaluate the liking and preference in a questionnaire. The high quality of the distributed fish was confirmed in a sensory profiling using a trained external panel.

	Thawed	Frozen
+ information	50	50
- information	50	50

Table 1. Test design. Fillets (thawed or frozen) were distributed in sealed plastic containers. 50 persons in each group have to return the questionnaire to make the test valid. Half of the persons got information about freezing and storing (high quality) or information about thawing. The persons getting thawed fish without information might had believed that the fish was unfrozen.

Results and discussion

Focus group interviews:

The participants discussed their attitude to packaging of frozen fish, preference of fish product, reliability of shopping place (table 2), consumer quality test and quality of frozen fish. Short resume of the positive and negative associations to frozen fish is given in table 3.

Table 2. Trust and reliability. Statements from the participants in the focus groups.

In general, the consumers do not trust the retailer
"Frozen fish is just another product"
Doubts about the retailers' knowledge
"Some consider themselves to be very expert, whereas others believe only in good prices and deals"
The fishmonger is perceived as being more quality-conscious
"He is more interested in quality, because that is his way of surviving in competition with the supermarkets"

Table 3. 'Emotional' statements about frozen fish from the participants in the focus groups.

Positive	**Negative**
"I think it is the 5 minutes in the microwave"	"I would prefer fresh fish, any time"
"For children it is an advantage that there are no bones in it"	"Taste doesn't remain, really not good"
	"It is difficult to see what quality it is"
"When you are in a hurry -It's quick"	"You have to thaw it before use. That takes a long time"
"It's cheap"	"The disadvantage with frozen fish: the taste"
"Better to buy frozen fish than frozen pizza"	"Frozen fish…they all taste the same to me"
"It is convenient to have it in the freezer, in case you suddenly want to make a particular dish"	"It really isn't the great gastronomic experience"
	"If you were barbequing, you would never consider buying frozen fish"
"You can buy it from many places"	

Focus groups yield results that have a qualitative nature. The results can not be projected to a larger scale as other quantitative methods are. They give, however, in-depth information on specific topic areas that can be useful in understanding the consumers attitude. Frozen fish is believed to be poorer than fresh fish

"I would prefer fresh fish, any time" especially due to the taste. The consumers prefer to buy fish at fishmongers, as they are perceived as being more quality-conscious.

Testing of frozen fish bought at retailers:

TVB-N: Code 1-20 ranged from 2.37 to 30.29 mgN/100g. Ref1: 2.38 mgN/100 g sample and ref 2: 1.95 mgN/100g sample. 6 of the samples had a TVB-N of more than 10 mgN/100g sample indicating that the fish was of poor quality before even frozen.

Free formaldehyde: Code 1-20 ranged from 1.2 to 40.3 ppm. Ref1: 1.4 and ref2 4.6 ppm. The two reference samples had the highest water holding capacity (3.55 and 3.28g water/g dry matter) and lowest thawing drip (7.4 and 2.1%). Results from chemical and physical testing were confirmed by sensory profiling where the assessors found a low fishy smell and taste, a low taste of freeze house of the reference samples. The reference samples and a few of the samples bought at retailers where found to have juicy texture with a low level of toughness and fibrousness.

Testing of frozen fish bought at retailers showed an unacceptable quality of many of the samples. The unacceptable sensory quality gives an explanation of consumers preference for fresh fish and attitude towards frozen fish as shown in the focus group test.

In- home test:

Profiling by the trained panel gave low scores for amine and fibrousness and high score for marine, sweet, flaky and juicy indicating a high quality fish.

59 % of the consumers returned the questionnaire. On a 9 point like/dislike scale the consumers scored between 8 and 9 with no significant difference between the four groups in the design. When asked about quality of the fish there was significant difference between the group given information that the fish was thawed (lower

score) and the other three groups although all four groups scored higher than quality of fish from retailers in general.

Conclusion

The focus groups revealed that consumers consider quality of frozen fish to be associated with lesser eating quality than fresh fish but at the same time convenient and less expensive. Testing of frozen fish bought at retailers showed an unacceptable quality of many of the samples and gave some explanation of consumer's attitude to frozen fish. The in-home test showed that the consumers liked frozen fish of high quality and that they preferred frozen fish to thawed fish and to the fish they normally buy.

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P 18. C-QIM A TOOL FOR CONSUMER EVALUATION

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Abstract

Development of a C-QIM (Consumer-Quality Index Method) has its offspring in the Quality Index Method (QIM) that has been developed in Europe in the last decade. The QIM is based on a non-destructive sensory assessment where a selected number of parameter is assessed on a standardized way. The C-QIM is a simplified scheme, which allows the consumer to make a sensory test when the fish is brought.

Introduction

The consumer often believes that the wet unwrapped fish they buy, always is fresh, ex. newly caught, but that is, unfortunately, not always true. To give the consumer a sensory tool to evaluate the freshness of the wet fish they purchase, a Consumer Quality Index Method (C-QIM) based on the QIM has been developed (Nielsen *et. al.* (2002)). Warm (2000) tested a C-QIM on a small grope of consumers at Danish Institute for Fisheries Research, Department of Seafood Research. The C-QIM was constructed with three sensory quality parameters for the following five fish species herring, cod, trout, flounder and saithe. The parameters where appearance, odour and texture, and to make the evaluation as simple as possible all were scored from 0-2 demerit points. Since consumers without any training should use the scheme, it was made user-friendly. This was done by giving a short descriptive instruction for each of the parameters in the scheme. In addition the C-QIM scheme was non-species specific, in contrast to QIM schemes (Hyldig and Nielsen (1997), Martinsdóttir *et. al.* (2001)), so in this way consumers could use the same scheme for all five species. Warm (200) showed that the C-QIM was workable for the consumer and that there were a correlation between C-QIM and the consumers liking of the fish. Disliking gave high C-QIM scores.

In the next step a new C-QIM scheme will be developed with several more species and in the autumn 2003 it will be tested at selected fish retailers in Denmark.

Conclusions

The long-term perspective of this kind of consumer information is that it may have a positive impact on consumer demand for fresh fish and the fish consumption when the consumer is better equipped for making an evaluation of the product that they are buying.

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Scientific bases for international standards on quality of fish products.

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L 26. THE INTELLECTRON FISCHTESTER VI – A NEGLECTED AND UNDERESTIMATED POWERFUL INSTRUMENT FOR FISH FRESHNESS/SPOILAGE DETERMINATION

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Abstract

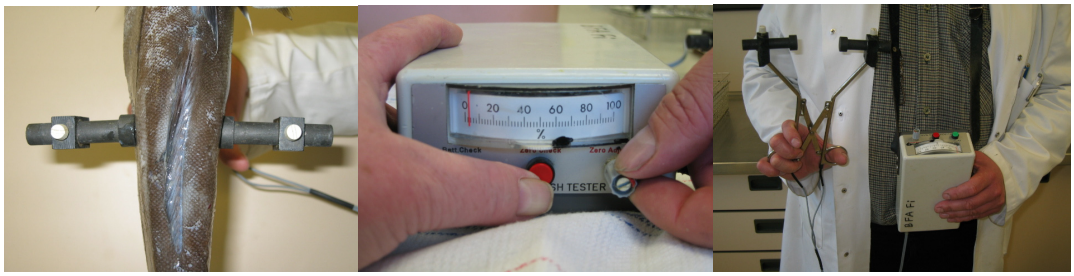
The measurement of the electric properties (resistance, conductivity, capacitance) of the fish flesh by transversal measurement through the fish body by the Intellectron Fischtester VI gives reliable information about the days in ice past and/or left of iced stored fish. The advantage of this instrument is that the decrease of the instruments readings is linear from day of harvest/catch to the date of spoilage (unfit for human consumption). The instrument was tested in a European Research Project (MUSTEC) together with a number of other instrumental methods. The Fischtester readings gave the best correlation with days on ice of all instruments tested. It performed as reliable as the sensory method used for calibration, the QIM Quality Index Method. Day in ice past or left can be predicted by Fischtester measurements for less than 1 day in ice.

The Fischtester readings can be used as an objective criterion for the state of freshness/spoilage together with sensory data across the fish chain. It offers also a potential as an instrument for traceability issues.

Introduction

To determine fish freshness quality, rapid, non-destructive and -invasive methods, which can be used by less educated personnel after minimal training, are sought by fish industry, authorities, fish inspectors and research. Measuring the electric properties of fish either transversal to the body axis offers an unique tool which fulfils all the demands for an ideal method (Oehlenschläger, 2003).

The basis for Fischtester measurements is that the resistance of absolutely fresh fish immediately after catch amounts to about 2 000 Ω, while stale fish close to spoilage has only 50 Ω. The conductivity of fresh fish is approx. 500 µS and that of old fish is around 20 000 µS.



In 1963 the development of a practical application based on the dielectric properties of fish was achieved. The conductance of fish tissue changes more rapidly than their dielectric properties and simple ac-measurements of impedance are not satisfactory. The Intellectron FISCHTESTER VI overcame this problem, and that of polarisation by measuring purely capacity changes from a simultaneous determination of impedance at two different frequencies (Hennings 1963, Hennings 1964). The Intellectron FISCHTESTER VI measures transversal through the entire fish, the electrodes are applied on the lateral line close to the anal opening, the readings range from 0 to 100, in a few species rarely above 100. The FISCHTESTER VI is equipped with an analogue indicator, the latest version VIa with a digital one.

Results and Discussion

A prerequisite for using instruments like the Fischtester is sound knowledge about basic values of readings as typical for freshly caught fish. Change in readings and readings found in unknown samples can only be assessent with this knowledge. In the years 1981-1985 these basic data have been collected during numerous cruises with research vessels. Table 1 gibes information on the Fischtester VI readings being typically for freshly caught marine species immediately after hauling.

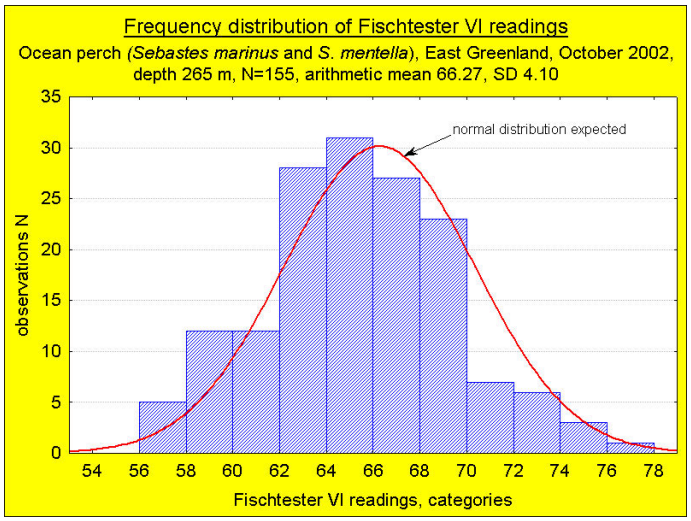
Table 1: Basic Fischtester data of different species from North Atlantic waters, all data measured on freshly caught fish (< 1 hour *post mortem*)

Species	Year	Catching area	N	**Arithmetic mean**	Median	Min	Max	SD
Ocean perch [1]	1984	WBW [a]	98	67.72	67	59	87	5.61
Ocean perch [1]	1984	WBW [a]	99	65.68	66	57	74	3.56
Ocean perch [1]	1984	WBW [a]	100	69.96	70	60	80	3.58
Ocean perch [1]	1984	WBW [a]	100	68.06	68	57	78	3.53
Ocean perch [1]	1982	East Greenland	108	66.92	66	59	79	4.31
Ocean perch [2]	1984	WBW [a]	100	76.16	77	56	94	7.98
Ocean perch [2]	1982	East Greenland	101	71.50	72	58	80	4.92
Ocean perch [2]	1981	WBW [a]	81	43.91	44	32	54	5.56
Ocean perch [2]	1982	Barents Sea	99	74.35	74	66	86	3.67
Ocean perch [3]	2002	East Greenland	155	66.27	66	57	78	4.10
Cod [4]	1984	WBW [a]	100	78.86	79	70	90	3.21
Cod [4]	1984	WBW [a]	74	74.61	75	62	87	5.27
Cod [4]	1982	Barents Sea	108	76.88	77	64	85	3.72
Haddock [5]	1984	Rockall Bank	100	85.71	86	68	100	4.68
Haddock [5]	1982	Rockall Bank	100	87.54	88	74	98	4.77
Haddock [5]	1982	Rockall Bank	57	80.98	82	59	93	7.79
Saithe [6]	1984	Rockall Bank	101	68.71	68	44	76	4.68
Blue Whiting [7]	1983	WBW [a]	99	84.90	86	68	99	5.32
Grenadier [8]	1981	WBW [a]	100	52.42	53	34	63	5.39
Grenadier [8]	1983	WBW [a]	100	57.84	58	34	72	6.83
Grenadier [9]	1983	WBW [a]	99	42.48	42	30	64	5.54
Black Scabbard-Fish [10]	1981	WBW [a]	56	88.34	88	74	98	5.19
Herring[11]	1983	St. Kilda	100	72.08	72	54	80	4.51

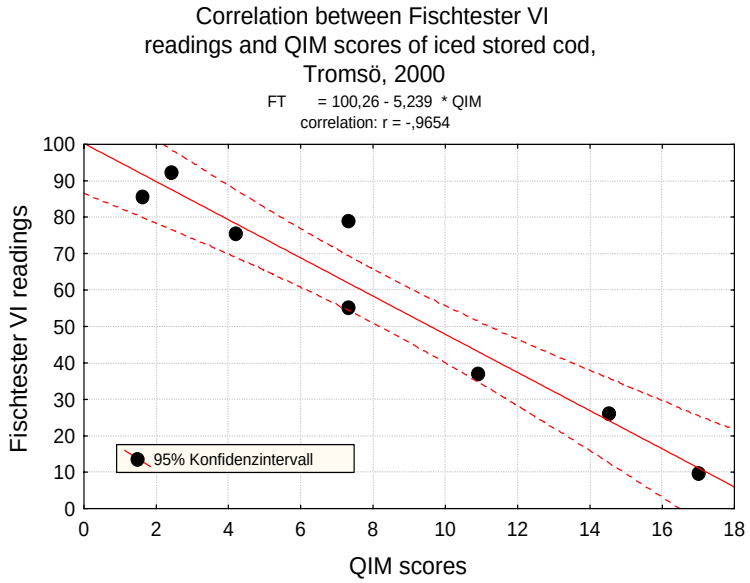
[1] *Sebastes marinus*, [2] *S. mentella*, [3] *Sebastes marinus* + *S. mentella*, [4] *Gadus morhua*, [5] *Melanogrammus aeglefinus*, [6] *Pollachius pollachius*, [7] *Micromesistius poutassou*, [8] *Coryphaenoides rupestris*, [9] *Macrurus berglax*, [10] *Aphanopus carbo*, [11] *Clupea harengus*

[a] Westbritish waters

The average values are scattering between species but are quite stable within a species, showing that e.g. cod and haddock have always very high initial readings while others like grenadier and ocean perch exhibit lower ones. The FT readings in a group of fish caught together are normally distributed (Fig 1):



The QIM method is the most promising sensory method for predicting days in ice left and days in ice passed of iced stored fish. In a series of experiments results obtained with Fischtester VI were compared with those obtained with the QIM method. The correlation coefficients of one experiment between days in ice and QIM scores and Fischtester readings were 0.9873 and 0.9901, respectively. The correlation between Fischtester VI and QIM scores was –0.9654 as shown in Fig. 2.



Since Fischtester measurements are based on the existence of intact cell membrane bilayers they fail when the cells are disrupted or broken. In frozen/thawed fish where no intact cell membranes have survived the freezing/thawing cycle Fischtester readings are always 0 because of very high conductivity. This phenomenon was used to differentiate fresh fish from frozen/thawed fish which was offered as fresh. Frozen/thawed fish would be rated very good by QIM but Fischtester measurement show very reliable and instantly that a perfect looking fish with FT readings of 0 has undergone a freezing(thawing cycle.

Conclusions

In numerous publications the Fischtester has shown its excellency in measuring fish freshness or degree of spoilage in iced stored wet fish. Here it could be shown that the reliability of the Fischtester measurements which takes only seconds and needs no special training is at least of the same quality as the QIM scores assessed by a panel or trainied specialists using a sensory method.

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L 27. INTERACTIONS BETWEEN SMOKING PROCEDURE AND RAW MATERIAL OF FARMED ATLANTIC SALMON (*SALMO SALAR*)

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Introduction

Atlantic salmon farming is of great economical interest to Norway and 40-50% of the quantum produced is cold-smoked. Numerous procedures for cold-smoking and processing are in use in various smokehouses. Occasionally, problems related to severe muscle gaping, soft texture and discoloration are observed. Deviant colour and soft texture have been related to the fat content of the fish (Rørå et al. 1998), and concerns regarding the quality of salmon with high fat content for smoking have been expressed by the industry (Sheehan et al. 1996; Espe et al. 2002; Espe et al. 2001). Little information is available on effects of processing parameters on quality characteristics of the smoked product and interactions with raw material properties. Thus, discrepant opinions prevail between salmon farmers and the smokehouses with respect to the suitability of the raw material for processing and the influence of processing method on the quality of the smoked product. Documentation of these factors is therefore of great importance to maintain a high quality production of smoked salmon with different raw materials, and to reduce future disputes with respect to causality.

Materials and Methods

Atlantic salmon (weight from 3.30 to 3.68 kg) from seven different commercial farms, geographically located in Norway and the Faeroe Islands, underwent processing procedures to produce cold-smoked fillets either according to a tough (mechanical filleting, injection-salting, extended drying time) or a gentle (manual filleting and dry salting) method, respectively. The salmon were either dry-salted for 18 hours at 4^{o}C (gentle) or injected with brine (25 % NaCl; tough), before the fish were dried and smoked (480-600 min, 22-23°C). The relative humidity was about 70 % during the process. The quality parameters investigated were processing yields, colour (CIE 1976 L*a*b*), carotenoid concentration, texture, muscle gaping, liquid-holding capacity, and TBARS (2-thiobarbituric acid reactive substances), phenol and salt contents. Data were statistically analysed by one-way and two-way analysis of variance (ANOVA) with interaction using the SAS computer software (SAS, 1996).

Results and Discussion

The total carotenoid content in the raw material ranged from 8.0 to 10.7 mg/kg. The major carotenoids present in the fillets were astaxanthin and canthaxanthin, and the latter comprised from 0-39 % of the total amount. The fat content ranged from 15.6-21.1 %. Small differences were observed in colour, gaping score and total liquid loss.

The process yields were about 14% higher for the brine injected than the dry salted fillets, approximately 103 vs. 90%, respectively ($p<0.001$). Similar post-smoking yields were obtained previously for salmon fillets using brine injection technology (Birkeland et al., 2003). Total liquid losses were higher in some of the groups treated with the gentle method, and could be attributed to a higher loss of lipid rather than water. It should be noted that these groups were from raw material with the lowest lipid levels.

The gaping score was on average 1.6 units higher (32 %) in brine injected than in dry salted fillets. The texture was firmer in the dry salted fillets. In general brine injected fillets were lighter (L*) and more yellow (b*) than dry salted fish, whereas redness (a*) was similar. Phenol and salt contents were not affected by processing method.

With the exception of process yield, the differences in quality parameters of the raw material were reflected in the cold-smoked salmon, and raw material characteristics affected the quality more than the cold smoke processing parameters used in this study. The relative difference between the groups was more pronounced for the salmon smoked by the gentle than the tough method. In conclusion, processing method profoundly affects the process yield. However, the quality of cold-smoked salmon was mainly affected by the chemical composition and quality of the raw material.

Acknowledgement

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L 28. A METHOD TO ASSESS *RIGOR MORTIS* IN FISH

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Abstract

Farming fish give a possibility to offer high freshness of products. Taking advantage of freshness by pre-rigor filleting has not been common in industry. Such products have been regarded as different, not satisfying the expectations for traditional quality of a fish fillet. Markets have changed and today it is of interest to influence the slaughter process and be able to produce a pre-rigor fillet.

Several factors influence the onset and strength of *rigor mortis* in fish; species, temperature, pre-slaughter handling and killing method (slaughter stress and exhaustion). Time before onset of *rigor mortis* decides if pre-rigor filleting is possible or not. In order to decide which parameters affects *rigor mortis*, it is a need for an objective and reliable method for measuring its onset and development. *Rigor mortis* is usually measured by indexes describing degree of bending the tail. Repeated measurements on the same fish are biased by the weight of the fish and handling, and a high number of fish should be measured. A more objective compression test has been developed and tested on several fish species. The new method allows the development and strength of *rigor mortis* to be measured in individual fish in a non-destructive and objective way. The new method uses a mechanical testing machine with specified running procedures.

Introduction

Farmed fish from Norway, mainly Atlantic salmon, are usually exported as iced, whole, gutted fish the same day it is slaughtered. Taking advantage of freshness by filleting fish pre-rigor has not been common in industry. It has been regarded as not being in demand in the market, representing a fillet with different quality due to contraction of fillets and difficulties in removing pin bones. When distributing whole fish much more weight than what finally ends up as the edible portion is transported. As long as most of the farmed Atlantic salmon is used for smoking fillets, the need for distributing the whole fish can be questioned. The backbone, skin and head become a problem of pollution for the buyer, while it is a potentially interesting by-product at the local slaughterhouse. New markets are now emerging for the strategy of distributing pre-rigor fillets, offering a “different” quality of the fillets. It is firmer, have a different shape with less gaping and has high freshness. Atlantic salmon fillets also have a more red appearance, due to a change in surface during the contraction.

Materials and Methods

The method allows for repeated measurements on the same fish without altering the development and strength of *rigor mortis*. It has been tested on halibut *(Hippoglossus hippoglossus*), salmon (*Salmo salar*), trout (*Oncorhynchus mykiss*), cod (*Gadus morhua*), saithe (*Pollachius virens*) and plaice (*Pleuronectes platessa*). The fish, approximately 2–5 kg, is placed on a plate and can be kept without handling in a storage room with temperature control in between measurements while *rigor mortis* lasts. The standardised compression test developed resembles the sensory “finger test”. Each measurement takes a few seconds. A mechanical testing machine (KGS) measure the force (gram) needed when pressing a sphere made of steel, (9,5 m.m. in diameter), a given distance (5 m.m.) with a constant speed (1 m.m/sec.), into the skin of the fish along the lateral line.

Results and Discussion

The compression force is dependent on the state of *rigor mortis*, depth of penetration and the location for measurement. When location and penetration are given, the compression force will indicate the state of *rigor mortis* in the fish. Recordings over time are presented as a curve by the computer in the mechanical testing machine.

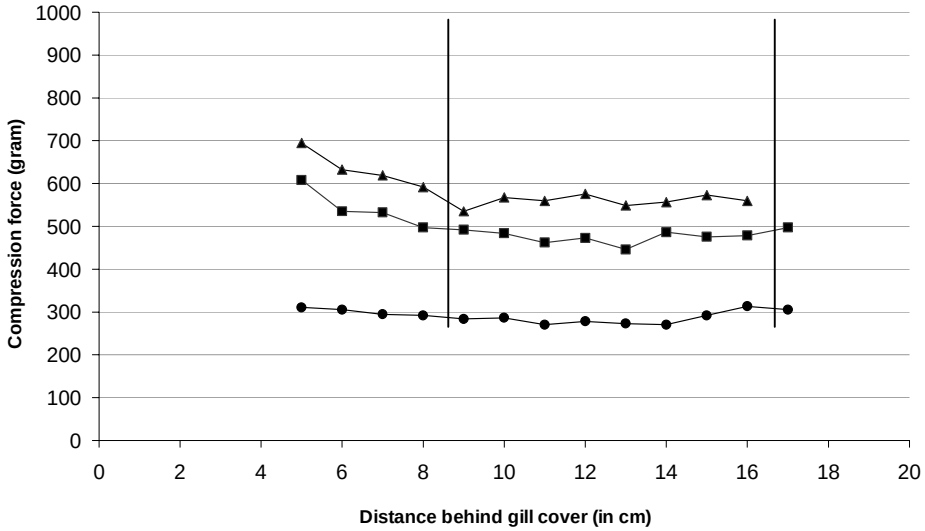

Figure 1. The difference in compression force when penetrating 5, 6 or 7 mm. into the skin along the lateral line of Atlantic salmon, is given for positions along the lateral line. ◆ 5 mm ■ 6 mm ▲ 7 mm

A penetration of 5 mm. is proposed for salmon as this results in stable recordings along a given length of the fish. 5 mm. penetration depth should also be used for trout, halibut, cod and saithe while on plaice 4 mm. is recommended.

The location where recordings are made is also critical. When measuring on positions within a specified area, we obtain consistent results giving an average value with small standard deviations. For Atlantic salmon this area is proposed as each cm from 6 to 16 cm behind the gill cover. This is the case in all three states of *rigor mortis*, resulting in readings that clearly distinguishes between the three stages of rigor mortis, pre-, in- and post-rigor, figure 2.

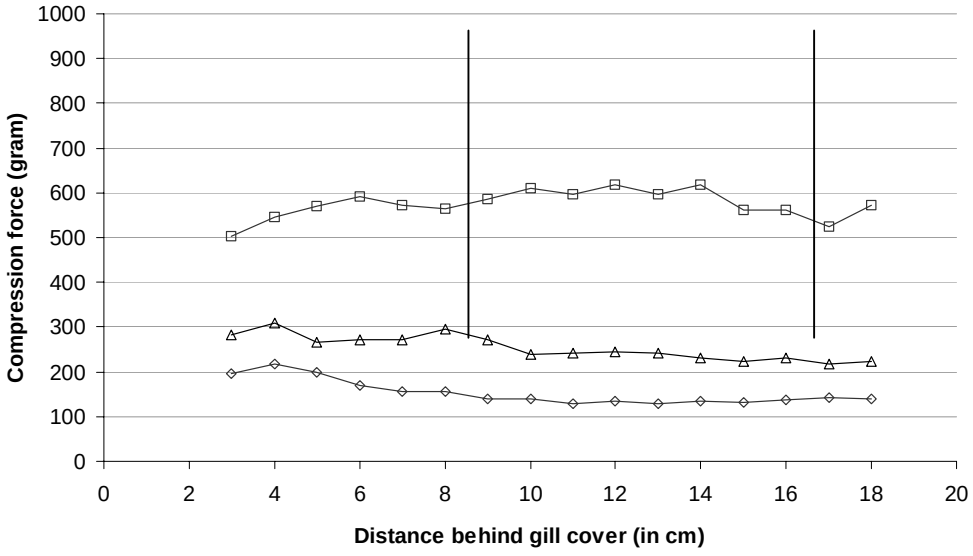

Figure 2. Compression force at 5 mm. penetration on A. salmon when fish is ◇ pre-rigor, □ in-rigor, △ post-rigor as function of position.

For Atlantic halibut the measuring area is from 1 cm to 12 cm behind the gill cover. For Plaice it is from 6 cm to 16 cm behind the gill cover along the lateral line. Cod and saithe are measured from 6 cm to 16 cm behind the gill cover.

A few measurements in one position are also comparable to the results obtained when changing positions along the lateral line. This allows for a high number of recordings on each fish, first from position 6 cm behind the gill cover until 16 cm, and then the same area again if needed. No difference was registered between right and left side of the fish. Location is independent of killing method i.e. handling at slaughter is not critical as long as the proposed area is used.

The shape of the curve obtained for compression force during storage is different depending on the slaughter method; exhaustion and killing, for the fish. The standard deviation between fish is greatest when the fish are entering *rigor mortis*, due to individual energy status of different fish. The method (compression force) distinguishes well between fish being in the pre-rigor, in-rigor or post-rigor state.

Conclusions

Onset and development of *rigor mortis* in different fish species can be measured in a non-destructive and objective way by a compression test. Using a sphere that is pressed a given, short distance into the skin of the fish in a specified area, a compression force is recorded by the computer in a mechanical testing machine. The area and penetration distance for measurement is slightly different dependent on fish species. When increasing depths the standard deviation increases, especially during the period when the fish is in-rigor.

The new method gives reliable measurement of *rigor mortis* development during storage. It correlates well with level of exhaustion for the fish at slaughter, as indirectly documented by development of pH (spear electrode) in the same individual fish during 4-6 days.

A hand held measuring device (RCFI-Penetrometer) uses the same principle. It is reliable and can be used in the field, but as presentation will show, it is more dependent on the operator.

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L 29. SENSORY PROPERTIES OF HERRING – INFLUENCE OF FISHING GROUND AND SEASON

(Not to be quoted without prior reference to the authors)

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Abstract

Herring (*Clupea harengus* L.) is traditionally considered as belonging to geographically well-defined stocks or populations, each with its characteristics including migration patterns. However, biological surveys have shown considerable mixing between populations to occur during migration. The fat content in herring varies according to the cycle of feeding and spawning. The fat content can vary considerable in batch of herring caught simultaneously, maybe due to the mixing of different herring stocks. Therefore herring caught at the same fishing ground and season can have different biological origin and different biochemical and functional properties as raw material. Little is known about the influence of this variation on the sensory properties of marinated herring, one of the traditional herring products in Denmark.

The objective of this study is to describe the sensory properties of marinated herring produced of raw material from different fishing grounds and seasons. Focus is on the correlation between difference in biological parameters of the raw material and the sensory properties. The sensory profile is also related to biochemical and functional properties.

Materials and Methods

Herring was collected on cruises in 2001-2002 by research vessel Dana in the North Sea, Kattegat, Skagerrak, and the Baltic Sea (Table 1). All samples were prepared onboard immediately after catch. The left fillet was frozen and stored at –80°C prior to the analysis of biochemical and functional properties. The right fillet was marinated in an acetic acid and salt solution and used for sensory analysis. All analyses are performed on individuals, i.e. on each caught herring excluding the variation between fish. A trained panel performed the sensory profiling on a 15 cm unstructured scale according to five odour-, six flavour-, and five texture descriptors over a period of 15 months and therefore a reference sample was included. Another trained panel evaluated the appearance of the fillets, e.g. colour and degree of gaping according to the principles in the QIM-system.

Results

The data were analysed by multivariate techniques allowing the optimal exploration and exploitation of data. The sensory analysis shows only little variation in the sensory properties. However there is a difference in flavour between herring caught in different fishing grounds (Figure 1). This effect is to some degree confounded with the effect of season. Herring caught in Skagerrak in July 2001 differ clearly from the rest and are characterised by higher scores for rancid and metallic odour and flavour. There is a tendency for herring caught in 2001 in the western North Sea in August and in the Baltic Sea in November to have a sweeter odour and flavour. Also there is a tendency for herring caught in 2002 in Skagerrak, the eastern North Sea, and Kattegat to have a more gritty texture, while herring caught in the eastern North Sea in July 2001 have a more fatty mouth feel than the rest.

An effect of fishing ground and season is seen on the appearance of the marinated fillets (Figure 2) caught in 2001. Herring from the Baltic Sea in November are characterised by having higher scores for bloodstains and yellow areas, while herring from the North Sea in August are characterised by having a more distinct silvery layer. Herring from Skagerrak and the eastern North Sea caught in July have more gaping and a darker colour.

The sensory profiles are related to other properties and the main effect resulting in the variation in sensory properties is the varying body weigh and fat content.

The importance of using reference material in the sensory evaluations is emphasised. The panel had a tendency to “drift”, but the introduction of a reference prevented drifting, and the panel was consistent in their evaluations both quantitatively and qualitatively.

TABLE 1. Details of samples used in the sensory analysis.

Year	Fishing ground	Season	N ICES-squares	N herring	Appearance	Sensory profiling	Abbreviation in figures
2001	Eastern North Sea	July	1	10	X	X	NSE/J01
	Skagerrak		5	86	X	X	S/J01
	Western North Sea	August	3	33	X	X	NSW/A01
	Baltic Sea	November	1	48	X	X	BS/N01
2002	Eastern North Sea	June/July	2	23		X	NSE/J02
	Skagerrak	July	7	66		X	S/J02
	Kattegat		3	19		X	K/J02

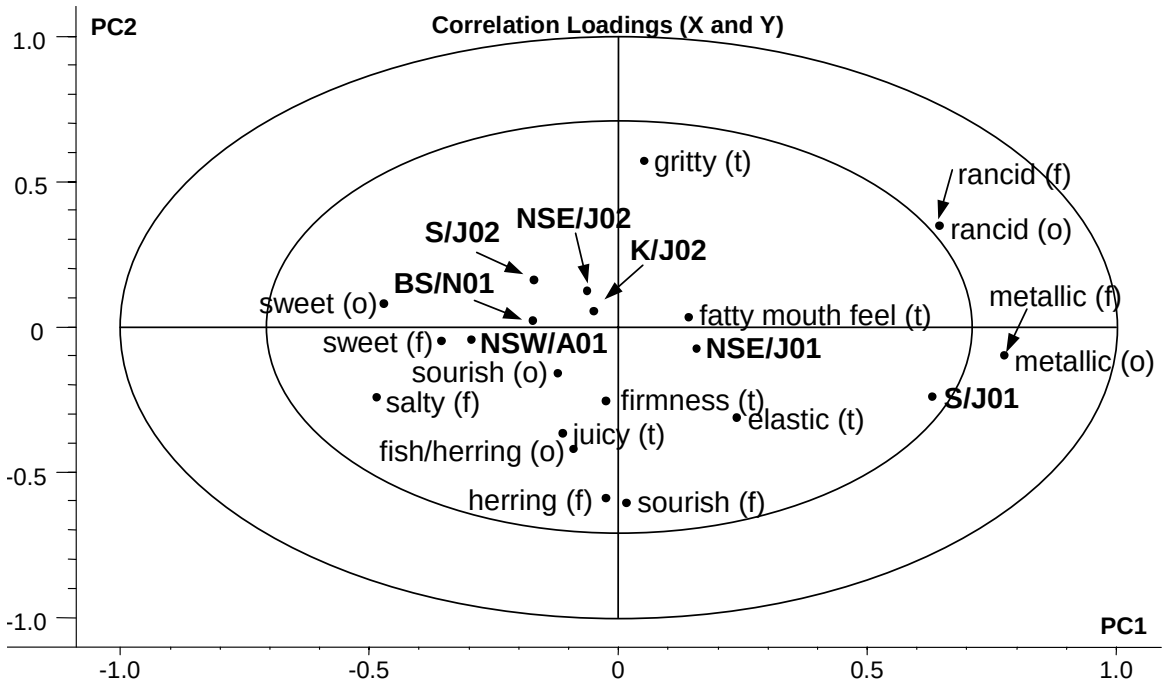

Figure 1. Correlation Loadings from a Partial Least Squares Regression model relating the sensory profile (X) to fishing ground and season (Y). The modelling is performed with full cross validation and weighted variables (1/stddev). PC1 explains 20% of X and 14% of Y, while PC2 explains 13% of X and 2% of Y. Details about samples are found in Table 1.

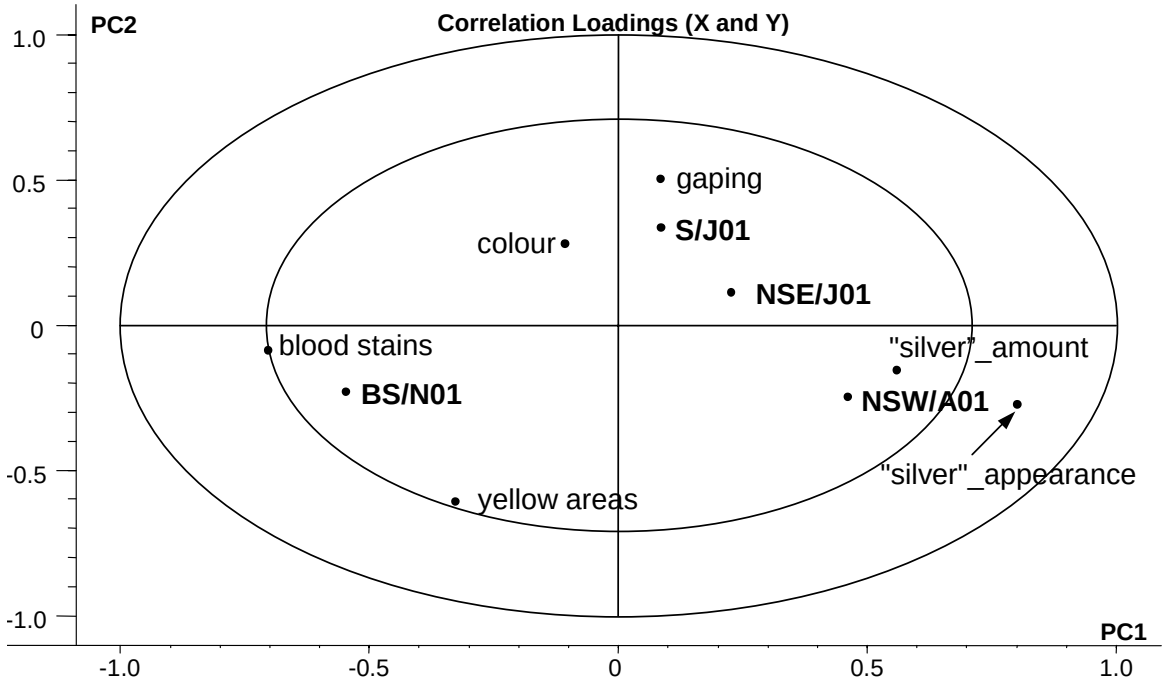

Figure 2. Correlation Loadings from a Partial Least Squares Regression model relating the appearance (X) to fishing ground and season (Y). The modelling is performed with full cross validation and weighted variables (1/stddev). PC1 explains 35% of X and 15% of Y, while PC2 explains 13% of X and 7% of Y. Details about samples are found in Table 1.

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L 30. CHANGES IN WATER-HOLDING CAPACITY OF HALIBUT MUSCLE DURING COLD STORAGE

(Not to be quoted without prior permission of the author)

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Abstract

A useful tool for describing quality in muscle foods post mortem is to measure the muscles water-holding capacity (WHC). Two storage experiments were carried out to study the development of WHC in halibut muscle during chilled storage for 15 and 18 days, respectively. To investigate the effect of normal spoilage bacteria on the WHC, the storage experiments were performed both in presence and absence of bacteria. Bacterial growth was inhibited by soaking the muscle in 3mM NaN_3 prior to the storage. A centrifugal technique was used to measure the WHC. Under normal conditions, the liquid loss (LL) initially increased followed by a decrease after approximately 8 days. When bacterial growth was inhibited, no such reduction in LL was observed before day 18. Under the normal storage conditions, no increase in muscle pH due to bacterial growth that could explain the improved WHC, was detected. The expelled water was analysed in order to investigate the mechanisms behind the changing WHC during storage. In both experiments, the total amount of protein lost from the muscle tissue correlated to the amount of LL. Hydroxyproline could only be observed in the LL from muscle heated to 50°C and no significant changes were observed during storage. This indicates that no degradation of collagen occurred during the storage period. Hexuronic acid was detected in the LL but no time dependent increase could be observed. In the normal samples, the amount of hexuronic acid in the LL actually appeared to decrease in the last part of the storage period. Gelatinolytic activities in the muscle tissue during storage were investigated by gelatine zymography. The results showed that in samples with spoilage bacteria present had higher gelatinolytic activities. The increased proteolytic activities are likely to result in more degradation of proteins including core protein of proteoglycans, which then contribute to the improved WHC.

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L 31. ON ESTIMATING THE WATER CONTENT OF DRIED SALTED FISH

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Abstract

There is a current need for industrial producers of dried salted fish to control the products' water content since some markets demand that the water content is below a defined upper limit. Traditionally, the water content is assessed by a trained person, a grader, in addition to spot testing of water content using a laboratory method. To be competitive, many producers of dried salted fish are now interested in replacing the manual grader by an instrument for measuring the water content of dried salted fish rapidly and non-invasively. Instruments that could fullfill such criteria have been evaluated and compared. Two companies provided dried salted fish (*Experiment 1:* 50 fishes from one company; *Experiment 2:* 70 fishes from the second company). The water contents of the fishes were measured gravimetrically; by point measurements or by cross-sectioning the whole fish and determination of the water content of each slice. Throughout *Experiment1* the fishes were also evaluated by a trained grader. Nuclear Magnetic Resonance (NMR) imaging was used to study spatial water distribution. The water content was also determined using several different measuring principles of near infra red (NIR) spectroscopy. It appeared that a manual grader can assess the mean water content of a dried salted fish with a prediction error of ±2%. Gravimetric analysis have a much lower (< 0.4%) prediction error. It is, however, unlikely that such a low prediction error can be arrived at by any relevant spot testing. NMR imaging shows that the water content varies throughout the dried salted fish. The correlation between water content determined at specific points and by the cross-section method was, however, fair. Using NIR instrumentation it was, at best, possible to determine the water content of each dried salted fish with a prediction error close to ± 1% using the cross-section method as reference method. Current commercial NIR instrumentation can estimate the water content of dried salted fish with a smaller prediction error than trained graders.

Introduction

There is a current need for industrial producers of dried salted fish to control the products' water content since some markets demand that the water content of dried salted fish is below a defined upper limit. Traditionally, the water content is assessed by a trained person; a grader. In addition, spot testing of water content using a laboratory method is carried out.

To be competitive, many producers of dried salted fish are now interested in replacing the manual grader by an instrument for measuring the water content of dried salted fish rapidly and non-invasively. The objective of this work was to investigate and compare different NIR instruments and principles with respect to their ability for rapid determination of water content of dried salted fish using the cross-section method (Codex Alimentarius, 2003) as reference method.

Materials and Methods

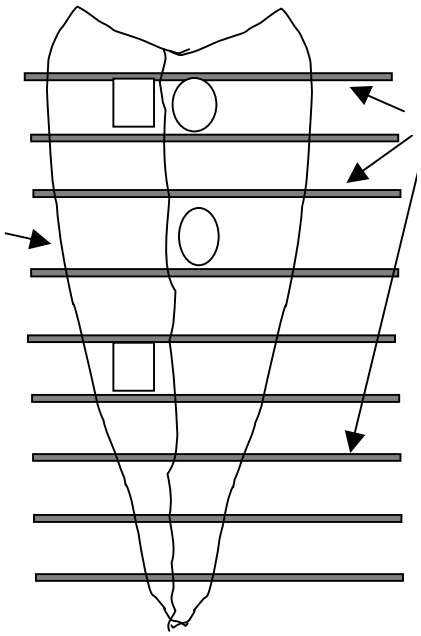

Figure 1. *Experiment 1: areas indicated by rectangles were used for moisture determination Experiment 2: NIR measurements were at elliptic areas.*

Raw materials: Two companies provided dried salted fish (*Experiment 1:* 50 fishes of water contents 38.7-54.2% from one company; *Experiment 2:* 70 fishes of water contents 38.3-49.2% from a second company.

Water content: The water contents of the fishes were measured gravimetrically (drying at 103-105°C for 24 hrs); by point/small area measurements or by cross-sectioning the whole fish and determination of the mean water contents of the sum of slices taken along the whole fish **(Figure 1).** Typical gravimetric moisture determinations (NMKL method 23, 1991) on homogeneous systems have a precision less than 0.4% (95% confidential interval). The cross-section method does not provide replicates, but we regard the precision to be somewhat larger than the above. The cross-section method has been indicated to give a good estimate of the true, mean water content of salted, dried fish (Codex Alimentarius, 2003).

For *Experiment 1* the fishes were also categorised by trained graders.

Spectroscopy/Imaging: Nuclear Magnetic Resonance (NMR) imaging was used to study spatial water distribution. Bruker's Biospec 2.4 Tesla (100 MHz) was used; sampling time was 5 min. Defined ^{1}H - concentrations in deuterium were used as water standards together with the fish sample within the coil. The relaxation times T_2 were used for image contrasts.

Several different measuring principles of near infra red (NIR) spectroscopy were used. These were: in transmission mode Infratec's Food and Feed Analyzer (850 – 1050 nm) and Zeiss OMK 500-H NIR from Zeiss group (945-1700 nm); in reflectance mode MM710 from NDC Infrared Engineering (8 filter instrument at 1490, 1630, 1728, 1800, 2100, 2180, 2230 nm) and in transflection mode NIRS 6500 from Foss Electric (780 -1100 nm).

Data treatment: Unscrambler version 7.6. (Camo A/S; Trondheim) for multivariate regression, Microsoft Excel 97 for general data registrations and MATLAB version 6.1 for probability analysis.

Results and Discussion

Analytical precision of spot testing:

A very rough estimate of the spot testing error was calculated by drawing 3 out of 25 fishes from a box (simulating a 42-44 % water content class as dermined by graders). The true mean water content of the box, requiring a 95% confidential interval, could not be estimated better than ±4% (**Table 1**). In practice, none will withdraw such a high fraction as 3/25 for spot testing. This illustrates that spot testing can only be used for specific purposes, and is unsuited as a method of securing a batch production of several tons.

Table 1. Number of withdrawn combinations (in %) that give a specific deviation from true (measured by cross-section method), mean water content of 25 dried, salted fishes in a box. Three fishes were withdrawn.

Deviation (+); in % salt content	1.5	2.5	4.5	6.0	7.0
Number of withdrawals (in %)	48.6	71.9	96.0	99.8	100

Analytical precision of graders:
Figure 2 shows the relationship between water content and the graders' classification into 5 water content classes. The root mean square error (RMSE) of deviation was 1.95%. The same RMSE was estimated from data obtained in *Experiment 2*.

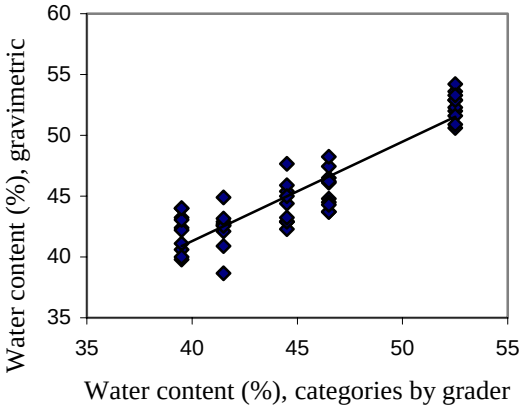

***Figure 2**. The relationship between water content categories determined by professional graders and water content determined gravimetrically at specific points (Experiment 1).*

Analytical precision of NIR:
Table 2 shows the result of the 4 methods used. Generally speaking the reflection method performed less well. If any of the two other measuring principles (transflection, transmission) were chosen, it would be possible to arrive at the same or a better precision than that of the graders. For the subset of 30 fishes *(Experiment 2)*, that had a nice distribution in water contents, both Infratech and NIRS 6500 would provide prediction errors below 1.5% (not shown).

Table 2. Prediction (using partial least square regression) of water contents of salted, dried fish using NIR-instruments from *Experiment* 2. NIR spectra were measured at two points and averaged (see **Figure 1** for definition). Reference water content was determined by the cross-section method.

Instrument	Correlation, r	Validated, predicted RMSE (%)	Number of principal components
Infratech	0.72	2.12	8
Zeiss*	0.78	1.46	9
NIRS 6500	0.76	2.25	6
MM710	0.56	2.74	4

* Only a subset of 30 fishes was measured.

Water content determinations in dried, salted fishes:
Figure 3 shows two sections of dried, salted fish of mean water content 63.6 % (Fig. 3a) and of mean water content 46.6% (Fig. 3b). The water content is generally higher where the fish is at its thickest. The water contents of areas/points used for regression (**Figure 1**, ellipses) do not correlate too well (r=0.67) to the water contents determined by the cross-section method; the water content is systematically overestimated at these two points compared to the cross-section method, and this puts recognizable constraints on the possibility for substantially reducing the prediction error. This also explains why better results have been reported in the transmission mode (Joensen and Wold, 1995) and on homogenous samples (Wold et al., 2003) using specific points for both chemical and spectroscopic measurements. This systematic deviation in water content determinations between specific points and the cross-section method needs to be reduced or better validated by using more or better measuring points/areas in future work.

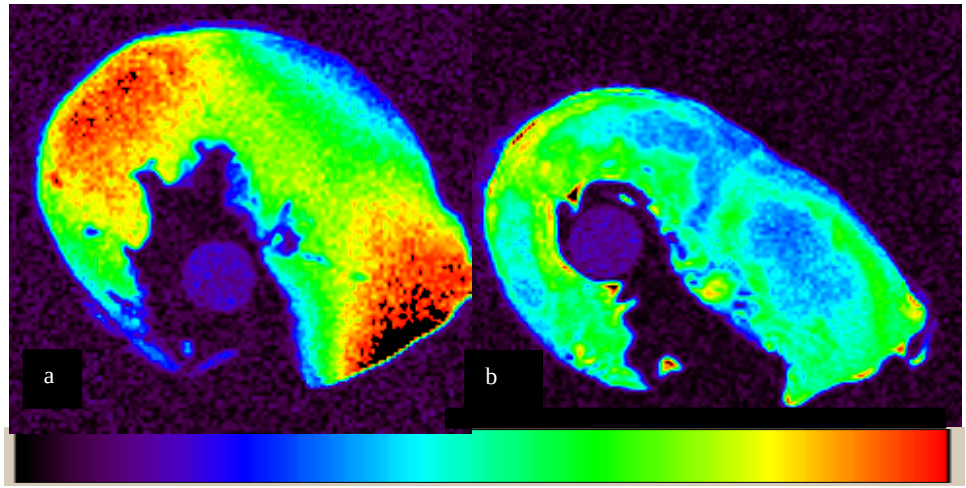

Figure 3. Proton magnetic resonance images showing inhomogenous water distribution in two salted, dried fish cross-sections (from *Experiment 1*). The circle (arrow) is the cross-section of a reference NMR tube (10 mm in diameter) containing 10 % H_2O.The water content decreases in the order: black → red-yellow →green→turquoise **or black→ light grey/whitish→medium grey→ dark grey if printed in black and white.**

Conclusion

Current commercial NIR instrumentation can estimate the water content of dried salted fish with the same or smaller prediction errors than trained graders. An instrumental, online method should preferably measure as much of the fish as possible since the water is unevenly distributed.

Acknowledgement

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L 32. DETERMINING WHICH FACTORS MINIMIZE RIME FORMATION WHEN TRANSPORTING LOOSE FROZEN SEAFOOD.

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Abstract

Too much rime in frozen seafood leads to deterioration in quality and lost sales. The objective of the research presented here was to determine how different factors influence the formation of rime in the process of transporting loose frozen shrimp and fish. Based on this knowledge it is possible to control more effectively these influencing factors during the transportation process and maximize the quality of the product.

To simulate real conditions, experiments were performed in freezer simulators. The first step in the research was to perform pre-experiments. The main purpose of those was to develop a method and a scale to measure rime that could be used in later experiments. Surprisingly few studies were found in the literature that discuss rime formation in frozen food, and a useful scale that could be applied to measure rime could not be found. Hence a method was developed to measure rime that is based on straining the rime from the seafood and weighting it.

The next step in the study was to perform systematic experiments to determine how different factors influence rime formation for loose frozen shrimp. To do this a design of experiments approach was used. Four factors were chosen; Base temperature (T), length of temperature change (L), size of temperature change (ΔT) and number of temperature changes (N). Design of experiments was used to determine which factors have significant effects on rime and to determine how various factors interact. Regression analysis was used to find a functional relationship between response variable and factors. All factors did have significant influence on rime but the factor number of temperature changes did influence rime formation the most.

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L 33. HOW FRESH IS THE FISH? EVALUATION OF FRESHNESS BY MEANS OF VIS/NIR SPECTROSCOPY.

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Abstract

Fresh fish is a perishable food; easily reduced in quality due to wrongful handling and storage conditions. With increasing consumer awareness of quality attributes and quality preferences, it is important to objectively measure and document the freshness state of fish. During storage the initial freshness of fish will decay due to the natural chemical and biochemical processes. By performing storage experiments of fresh cod (*Gadus morhua*) it has been evaluated how visible / near-infrared (VIS/NIR) spectroscopy can be used to determine fish freshness - or in other words: to determine the remaining shelf life.

The technique of NIR spectroscopy has been developed over the last 30 years to evaluate quality parameters in numbers of foodstuffs such as grains, fruits, meats and also fish. In this study, cod were stored for a period of 16 days. Throughout this period fish were taken out for VIS/NIR measurements and in addition microbiological, chemical, biochemical and sensorial analysis were performed. The measurements were evaluated by multivariate statistics in order to model a possible connection between spectroscopic recordings and the chemical / biochemical freshness state of the fish.

The VIS/NIR spectra from cod correlate with storage time. In particular it was seen that the visible part of the spectrum contribute the most to modeling the elapsed storage time. It was also shown a correlation between spectral recordings and the sensorial QIM score of the fish, hence VIS/NIR spectral measurements can be used to evaluate the remaining shelf life of cod according to the QIM-scheme.

VIS/NIR spectroscopy has thus proven useful for the evaluation of fish freshness. This method can meet requirements to speed and objectivity when measuring freshness.

Introduction

Fresh fish is a perishable food; easily reduced in quality due to wrongful handling and storage conditions. Shortly after harvest the fish is assumed to retain its original characteristics. During storage these characteristics will change until spoilage occurs due to biochemical, chemical, physical and microbial processes that are affected by both time and temperature (Ashie *et al*., 1996). With increasing consumer awareness of quality attributes and quality preferences it is important to objectively measure and document the freshness state of fish. Regarding further processing and how to best utilize the raw material, it is also of interest to know the freshness state. All in all one would want an easy, objective and low-cost method to determine the storage time of fresh fish.

Materials and Methods

Storage experiments with cod (*Gadus morhua*) were performed. Cod caught on long-line (overnight in the sea) were slaughtered, bled, gutted, cleaned and then put in ice. Subsequently on days 0, 1, 2 3 4, 6, 8, 10, 13 and 16 five fishes were taken out for measurements.

Sensory evaluation according to a modified QIM (Quality Index Method, Luten and Martinsdóttir, 1997) scheme was performed with a trained expert panel of three persons. Sensory evaluation was performed prior to filleting of the fish.

Visible / near-infrared spectroscopic measurements were made using the NIRS 6500 (Perstorp Analytical Inc., Silver Spring, Md., U.S.A.). The instrument was operated in fiber optic mode, performing the transflection measurements on the inside of the loin (Nilsen *et al*., 2002).

Data were analyzed by use of the software *the Unscrambler* 7.5 (CAMO, Norway). Partitial Least Squares Regression (PLS1) (Martens and Næs, 1989) was used to investigate the correlation between the spectral data and the freshness state of the fish.

Results and Discussion

As in previous work (Nilsen *et al*., 2002) it was found that the visual spectral range contained the most information regarding the freshness state of the cod. PLS1 analysis was performed on the spectral data set using the storage time as the response parameter. Figure 1 shows the measured versus predicted storage time for the cod samples. As seen from the figure the storage time is fairly well predicted from the measured spectra. A correlation of prediction, corrP, of 0,96 and an error value of prediction, RMSEP of 1,43 days describe the model. These model parameters are obtained without preprocessing of the spectral data or removal of possible outliers in the data set.

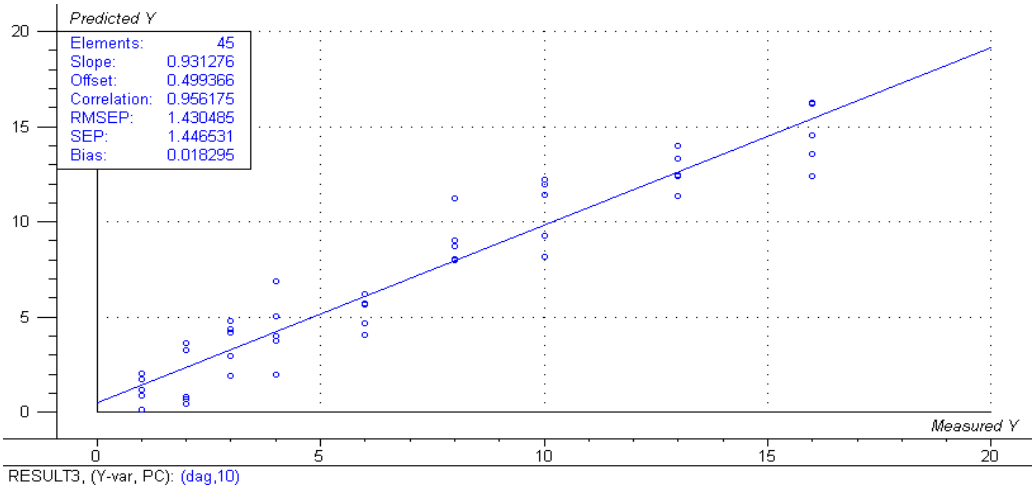

Figure 1 Predicted versus measured storage time of cod.

PLS1 was also run on the visible spectrum with the measured QIM value as the response vector. In Figure 2 the predicted versus measured QIM values are shown. Also for this regression analysis the model parameters obtained were considered to be good. The corrP was 0,96 and the error value RMSEP was 2,49 points.

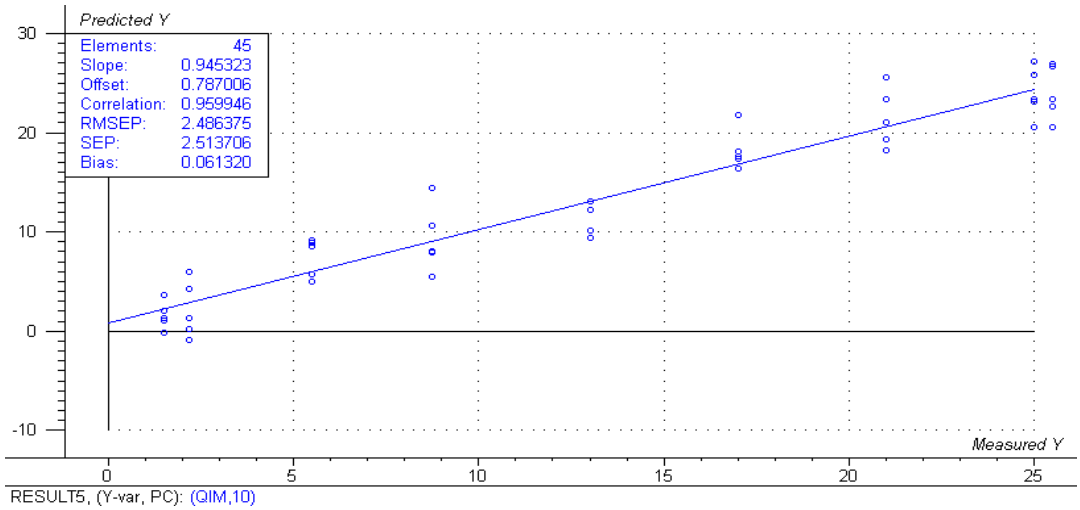

Figure 2 Predicted versus measured QIM score of cod.

The Quality Index Method describes a number of different parameters relating to deterioration of fresh fish. The finding that VIS/NIR spectroscopy correlates with the QIM score is very promising in view of finding a simple instrumental method that reports and quantifies the deterioration processes in fresh fish.

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L 34. A NEW APPROACH TO MEASUREMENT OF FRESH AND FROZEN FISH QUALITY.

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Abstract

A concerted action on the evaluation of fish freshness and quality under the 3rd Framework Programme of the European Community (AIR3 CT94 2283) examined in detail the various methods available amongst which were physical methods. However it was concluded that no physical methods were entirely satisfactory and that all needed to be significantly improved to fulfil the expectations of the users. The new dielectric method described in this paper tries to address these problems.

Recently, another European Communities funded project (FAIR CT97-3020) widened the scope of such dielectric measurements by using a combination of dielectric spectroscopy and multivariate analysis to determine not just other constituents of a material but also some loosely defined quality of foods. Thus for example, fish that had been frozen could be distinguished from those that had not, and different types of raw material for processing also could be discriminated.

This has led to a further project under the 5th Framework Programme (QLRT-2000-01643: SEQUID) designed to develop the method further for the determination of the quality of fresh and frozen fish. The preliminary work to be reported here concerns

- changes related to time of storage on ice of fresh fish (Baltic cod (*Gadus morhua*) and farmed North Atlantic salmon (*Salmo salar*))
- time/temperature effects in long term frozen storage of frozen fish (South Atlantic and Pacific Hake (*Merluccius spp*.) and Baltic cod (*Gadus morhua*)).

Variables related to the dielectric properties of individual fish were measured in the microwave region using an open-ended coaxial sensor and automatic network analyser. The multivariate data so obtained contained many highly co-linear variables. These were subjected to principal component analysis both to reduce the number of variables and to eliminate the problem of co-linearity. The principal components obtained were then used in multiple regression to produce calibration equations for the prediction of a number of variables of interest e.g. time of storage, temperature of storage, QIM and other sensory scores. In addition, using discriminant analysis on the relevant principal components, the fish were sorted into defined groups, e.g. frozen once or twice, days on ice.

Discrimination was generally achieved with high success rates. For example the success rate of assigning cod stored on ice to the correct storage time group was never less than 91% and for the salmon was 100%. As another example, twice frozen cod could be discriminated from once frozen with better than 90% success.

Introduction

Historically, the interaction of low power microwave electromagnetic fields with food materials has typically meant the measurement of water content. Recently, building on earlier work carried out at Torry Research Station in Scotland (Kent and Anderson 1996), a European Communities funded project (FAIR CT97-3020) widened the scope of such dielectric measurements by using a combination of dielectric spectroscopy and multivariate analysis to determine the proximate composition, including added water, of a number of food products (Kent *et al* 2000a). However, not just constituents of a material but also some loosely defined quality factors of foods could be determined. Thus for example, fish that had been frozen could be distinguished from those that had not, and different types of raw material for processing also could be discriminated (Kent *et al* 2000b). This work has led to a further project under the European Communities' 5th Framework Programme (QLRT-2000-01643: SEQUID)

designed to develop a method for the determination of the quality of fresh and frozen fish. Some of the preliminary work of this project will be reported here and concerns

- changes related to time of storage on ice of fresh fish (Baltic cod (*Gadus morhua*) and farmed North Atlantic salmon (*Salmo salar*))
- time/temperature effects in long term frozen storage of frozen fish (South Atlantic hake (*Merluccius capensis*), South Pacific hake (*Merluccius australis*) and Baltic cod (*Gadus morhua*)).

Materials and Methods

For the iced fish measurements Baltic cod and Atlantic salmon were acquired as soon as possible after harvest, gutted and stored on ice up to the end of their commercial life. At approximately 2-day intervals, samples were taken for dielectric measurement and assessment by the Quality Index Method (QIM).

Frozen cod of known thermal history were obtained by freezing fresh fish from suppliers and storing at temperatures of –10, -20, and -30°C. Some samples of cod stored at –20°C were thawed after two weeks, then refrozen and stored again at –20°C. The hake obtained from suppliers however had been frozen at sea and its history after catching was assumed to be one of frozen storage at –20°C. After acquisition they were stored as for the cod. At regular intervals over about one year samples were taken from cold store and thawed. Various analyses and sensory assessments were made on the samples as well as the dielectric measurements.

The dielectric measurements were made using the method of Time Domain Spectroscopy (TDS). TDS applies an electromagnetic step-pulse with a very fast rise time (100ps) to a sensor, typically an open ended coaxial line placed in contact with the material under test (MUT). The dielectric properties of the MUT distort the pulse, either in reflection or transmission. This distorted signal thus contains information on the dielectric properties and appropriate data analysis can use or extract such information. Variations are assumed to result from changes in the physico-chemical environment, principally of the water molecules in the tissue and various ionic solutes. These variations were analysed using principal component analysis (PCA) of the sampled TD pulse and regression of a number of variables of interest (e.g. time of storage, temperature, QIM, organoleptic properties) against the PCs (PCR) to deduce calibration equations. From these regression equations the adjusted coefficient of determination R^2_{adj} and the standard error of calibration (SEC) were calculated. The standard error of prediction (SEP) was calculated by a process of internal cross-validation (ICV). Linear discriminant analysis was also applied using the PCs as descriptors to group samples according to age, treatment, provenance etc.

Results and Discussion

Fresh Salmon and Cod

Full comparison of all the results can be seen in table 1. The different measurement strategies that were applied can also be seen.

Table 1 Prediction of time of storage and QIM for both fresh salmon and cod using principal component regression of 10 PCs. Standard errors are in the appropriate units

		Salmon			Cod		
		R^2_{adj}	**SEC**	**SEP**	**R^2_{adj}**	**SEC**	**SEP**
Fish fillet skin side	**Days-on-ice**	95.2	1.4	1.7	70.6	2.5	3.0
	QIM	88.7	2.1	2.5	67.4	2.2	2.8
Fish fillet flesh side	**Days-on-ice**	93.4	1.6	1.9	75.3	2.3	2.5
	QIM	82.1	2.6	2.8	75.3	1.9	2.0
Mince	**Days-on-ice**	92.7	1.7	2.0	85.9	1.7	1.9
	QIM	79.2	2.8	3.0	79.8	1.7	1.9

If the samples were placed into groups according to time of storage, then discriminant analysis correctly allocated each to its true group. This was for all measurement methods in both training and validation.

Frozen cod

Table 2 summarises the results on frozen cod. The prediction of temperature of storage can be seen to improve significantly if the twice-frozen fish are removed from the calibration. All sensorial scores correlated well with the PCA of the dielectric data. These scores on a scale of 1 to 100 were based on the following attributes; toughness, dryness, fibrosity, perceived age, fishiness, unpleasantness, cold store flavour, and overall influence of cold storage. For economy of space only the results for perceived age are included in this summary in table 2.

Table 2 Frozen cod: Prediction of time, temperature of storage, and influence of cold store sensory score for different thermal histories.

	Temperature prediction			Time prediction			Perceived age		
Sample	**R^2_{adj}**	**SEC**	**SEP**	**R^2_{adj}**	**SEP**	**SEP**	**R^2_{adj}**	**SEC**	**SEP**
All cod	41.1	5.0	5.4	89.8	32.3	34.9	77.6	11.7	12.7
Twice-frozen cod (stored at -20ºC)	-	-		97.0	17.5	17.3	-	-	-
Single-frozen cod	72.8	3.9	4.2	93.0	27.0	30.2	72.1	11.1	12.0

The discriminant analysis results separating once- and twice-frozen cod are shown in table 3. Since the calibrations are different for the two groups it is feasible to use such discriminant analysis to determine the prior treatment before using the appropriate predictive equations for time and temperature of storage.

Table 3 Frozen cod: Discrimination of once- from twice-frozen fish

	No. in group 1 (once-frozen)	85	
	No. in group 2 (twice-frozen)	25	
Training	Predicted group	**True group** **1**	**2**
	1	82	0
	2	3	25
	Percent correct	96.5	100.0
	Percent correct overall	97.3	
Validation	Predicted group	**True group** **1**	**2**
	1	81	0
	2	4	25
	Percent correct	95.0	100.0
	Percent correct overall	96.4	

Frozen South Atlantic (SA) and South Pacific (SP) hake

Two species of hake were studied: *Merluccius capensis* from the South Atlantic *and Merluccius australis* from the South Pacific. Both species were studied in the form of whole fish (gutted and beheaded) and *Merluccius capensis* also was obtained as frozen fillets. Although many variables known to change with cold storage were studied only a few are shown in the summary in table 4.

Table 4. Frozen hake: Prediction of some variables of interest

	Variable	**R^2_{adj}**	**SEC**	**SEP**
Whole fish (SA)	**Temperature of storage**	27.0	6.9	7.1
	Time of storage	99.2	24.9	25.3
	Demerit points (raw)	75.7	1.2	1.2
	Demerit points (cooked)	73.8	0.6	0.6
Whole fish (SP)	**Temperature of storage**	73.3	3.9	4.1
	Time of storage	86.7	25.6	29.3
Fillets (SA)	**Temperature of storage**	21.5	7.3	7.6
	Time of storage	91.2	24.6	26.3

Prediction of temperature is poor but this is not surprising since the Pacific hake were initially stored for up to about 150 days on board fishing vessels at –20ºC. The Atlantic hake was similarly stored for 56 days. The temperature predicted is therefore the effective temperature over the storage time of the fish and does not relate directly to the temperature of storage in the laboratory.

Discriminant analysis using the PCs as descriptors was able to assign samples correctly to the two species with a high degree of success, as table 5 shows. The same comments made above for cod, with regard to initial discrimination of samples would also apply here.

Table 5 Discrimination of species of hake used

No. in group 1 (South Atlantic)	70			
No. in group 2 (South Pacific)	50			
	Training		**Validation**	
Predicted group	**1**	**2**	**1**	**2**
1	67	1	67	5
2	3	49	3	45
Percent correct	95.7	98.0	95.7	90.0
Percent correct overall	96.7		93.3	

Conclusions

The results presented here are only a small fraction of the work that has been carried out so far. Nevertheless they show that this new method has great potential for providing many of the requirements demanded by the industry for quality determination by instrumental methods. The next phases of the project will see the construction of prototypes and their validation by the partners in this project.

Acknowledgements

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P 19. EFFECT OF CITRIC ACID ON RANCIDITY DEVELOPMENT IN HORSE MACKEREL (*Trachurus trachurus*) DURING FROZEN STORAGE

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Abstract

The present study was aimed to investigate the effect of a natural commercial antioxidant (citric acid; CA) for enlarging the shelf life during the frozen storage of horse mackerel (*Trachurus trachurus*). For it, previously to freezing and frozen storage, fish fillets were dipped in CA aqueous solutions (0.05%, 0.15% and 0.50%) and compared to water dipped (Water Control) and untreated (Blank Control) fillets.

An inhibitory effect of CA on rancidity development was concluded, specially in the case of the most concentrated pre-treatment (0.50%). This behaviour can be explained as a result of CA ability for quenching transition metals that are known to be present in the fish muscle. Water treatment (Water Control) of fillets also showed some inhibitory effect compared to Blank Control as a result of blood removal from fish fillet.

Introduction

The fish industry is actually suffering from dwindling stocks of traditional species as a result of drastic changes in their availability. Thus, fish technologists and fish trade have turned their attention to some unconventional sources of raw material. One of such species is horse mackerel (*Trachurus trachurus*), a medium-fat content fish abundant in the Northeast Atlantic (Vareltzis et al., 1997; FAO, 2002).

Frozen storage has been widely employed to retain fish properties before it is consumed or employed in other technological processes (Pigott and Tucker, 1987). However, during frozen storage, enzymatic and nonenzymatic rancidity is known to strongly influence the shelf life of relatively fat fish products (Erickson, 1997).

To extend lag phase as long as possible and accordingly, retard lipid oxidation during the frozen storage, a great attention is being given to the employment of natural antioxidants (Frankel, 1995; Decker, 1998). Citric acid (CA) and its salts are widely known for their role as chelators and acidulants in biological systems. CA has been proven to inhibit microbial growth, to improve physical and sensory properties in frozen fish products (Ponce de León et al., 1993; Chawla et al., 1996) and to play a synergistic role with primary antioxidants and oxygen scavengers (Stodolnik et al., 1992; Jaswir et al., 2000).

The present work concerns horse mackerel trading as a frozen product. The study is aimed to investigate the effect of citric acid on the stability of horse mackerel fillets during its storage up to 6 months. Lipid damage (oxidation and hydrolysis) assessment was carried out.

Materials and Methods

Fresh horse mackerel (*Trachurus trachurus*) were obtained 10h after catching. Upon arrival in the laboratory, the fish were carefully dressed and filleted by hand, divided into five groups and kept on ice. Individuals of the first group were left untreated (Blank Control), directly packaged in polyethylene bags and immediately frozen at –80ºC. The remaining four groups were immersed in the following four aqueous solutions: Distilled water (Water Control), 0.05 % CA, 0.15 % CA and 0.50 % CA. The fillets were removed after 5 min, packaged in polyethylene bags and frozen at –80ºC. After 24 hr at –80ºC, all fish fillets (treated and untreated) were moved to a –20ºC freezer. Sampling was undertaken after 1, 3 and 6 months of frozen storage at –20ºC and on the starting material (Initial values). Analyses were carried out on the white muscle. For each kind of treatment (both controls and the three CA solutions), three different fish batches were considered and studied separately to achieve the statistical study.

Lipids were extracted by the Bligh and Dyer (1959) method. Free fatty acids (FFA) content was determined by the Lowry and Tinsley (1976) method. The peroxide value (PV) was determined by the ferric thiocyanate method (Chapman and McKay, 1949). The thiobarbituric acid index (TBA-i) was determined according to Vyncke (1970).

Fluorescence formation (Perkin-Elmer LS 3B) at 327/415 nm and 393/463 nm was studied (Aubourg et al., 1998). The relative fluorescence (RF) was calculated as follows: $RF = F/F_{st}$, where F is the fluorescence measured at each excitation/emission pair, and F_{st} is the fluorescence intensity of a quinine sulphate solution (1 µg/ml in 0.05 M H_2SO_4) at the corresponding wavelength. The fluorescence ratio (FR) was obtained from the lipid extract (Bligh and Dyer, 1959) analysis, according to the following calculation: $FR = RF_{393/463nm} / RF_{327/415nm}$.

Data from the different lipid damage measurements were subjected to a one-way ANOVA ($p<0.05$) (Statsoft, 1994); comparison of means was performed using a least-squares difference (LSD) method.

Results

As a result of frozen storage, a gradual increase in FFA formation was observed for all samples, thereby indicating that hydrolytic activity of lipid constituents was occurring over time. Comparison among the different treatments did not show significant differences so that an effect of antioxidant treatment on lipid hydrolysis could be assessed.

According to primary (peroxides), secondary (thiobarbituric acid reactive substances) and tertiary (fluorescent compounds) lipid oxidation compounds assessment, citric acid treatment showed to inhibit the rancidity development during the frozen storage. Best oxidation inhibition results were obtained when employing the most concentrated CA solution (0.50%). Results on fluorescence detection are shown in Table 1.

Water treatment of fillets (Water Control) also showed some inhibitory effect compared to the Blank Control that can be explained as a result of blood removal from fish fillet, according to previous research (Richards et al., 1998).

An inhibitory effect of citric acid on rancidity development is concluded. This behaviour can be explained as a result of citric acid ability for quenching transition metals that are known to be present in the fish muscle. Further studies employing this antioxidant are recommended during the frozen storage of fatty fish species.

TABLE 1: Fluorescence ratio (FR) determination* during frozen storage of horse mackerel fillets that were pre-treated under different conditions**

Treatment	Initial Value	Frozen Storage Time (months)		
		1	3	6
Blank Control	^{w}0.17 (0.034)	^{w}0.25 a (0.044)	^{x}0.45 a (0.147)	^{y}1.59 c (0.042)
Water Control	^{w}0.17 (0.034)	^{w}0.24 a (0.057)	^{w}0.37 a (0.083)	^{x}1.36 bc (0.331)
0.05% CA	^{w}0.17 (0.034)	^{w}0.24 a (0.026)	^{w}0.32 a (0.052)	^{x}0.96 ab (0.187)
0.15% CA	^{w}0.17 (0.034)	^{w}0.25 a (0.082)	^{w}0.31 a (0.052)	^{x}0.75 a (0.163)
0.50% CA	^{w}0.17 (0.034)	^{w}0.20 a (0.044)	^{w}0.26 a (0.083)	^{x}0.60 a (0.202)

* Mean values of three independent determinations. For each row, means preceded by different superscripts (w-y) are significantly ($p<0.05$) different. For each frozen storage time, means followed by different letters (a-c) are significantly ($p<0.05$) different. Standard deviations are indicated in parentheses.

** Treatments: Untreated fillets (Blank Control), water treated fillets (Water Control) and citric acid (CA) treated fillets (0.05 % CA, 0.15 % CA and 0.50 % CA).

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P 20. CORRELATION BETWEEN SENSORY ANALYSIS AND DYNAMIC HEADSPACE GC ANALYSIS IN STORED MARINATED HERRING

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Abstract

Herring is susceptible to lipid oxidation due to its high fat content and high level of polyunsaturated fatty acids. Lipid oxidation results in formation of secondary volatile oxidation compounds (aldehydes, ketones, alcohols etc.) that will give rise to rancid off-flavour formation. Dynamic headspace gaschromatography is often used to determine concentrations of secondary volatile oxidation products in foods. Sensory and GC data have been reported to correlate well with each other in several studies on food systems containing n-3 fatty acids (e.g. fish oil, fish oil enriched mayonnaise). However, contradictory results have been reported in the literature with respect to correlations between sensory data and volatile data in fish. The objective of the present study was therefore to investigate the relationship between sensory data and dynamic headspace GC data obtained in stored marinated herring with particular emphasis on off-flavour formation caused by lipid oxidation.

Herring caught at different fishing grounds around Denmark were used. Some herrings were marinated immediately after being caught, while others were marinated after two or four days on ice storage. The herring was filleted and then marinated in a brine of 7% acetic acid and 16% salt. Subsequently, the marinated fillets were stored for a minimum of 30 days at 2^{o}C. Immediately before sensory evaluation the fillets were kept in a 1% NaCl-solution in 2 days to reduce acid and salty flavours in the fillets. A trained sensory panel then evaluated the odour and flavour profiles of the fillets. One fillet from each fish was used for sensory evaluation while the other fillet was used for dynamic headspace GC-FID analysis. Fillets for GC-analysis were frozen at – 80^{o}C after sampling until the analysis was carried out. Data were analysed by partial least squares regression analysis.

The data obtained will be discussed in relation to how the herring was treated after catch; i.e. marinating immediately after catch vs. marinating after 2 or 4 days on ice.

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P 21. DETERMINATION OF VOLATILE COMPOUNDS TO CHARACTERIZE FISH FRESHNESS

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Abstract

Food quality and safety are nowadays at a premium, and it is crucial to have reliable methods for assessment of the spoilage of fish, a highly perishable foodstuff.

Numerous methods (organoleptic/physical/chemical/microbiological) have been proposed, but all are limited in their applications, whence the need for novel approaches. Sensory methods define a freshness index by assessing appearance (skin, mucus, eye, gills…), odour, etc. These are effective when applied to whole fish by experienced operators, but are more problematical in the case of fillets or processed products, for which there are fewer observable parameters. Our approach is akin to that of sensory methods since it identifies the molecules that account for the odour of decomposed fish. Indirect organoleptic assessment of samples makes use of chromatographic methods to recover volatile odour compounds.

The data obtained by comparison on two levels of conservation (Day 0 and Day 10) per HS-MS analysis and SPME-GC/MS analysis are presented. HS-MS analysis made it possible to highlight 19 ions which could be retained as marker of fish freshness. In SPME-GC/MS, 86 compound was identified and 20 compound could be possibly retained to characterize the freshness of fish, mainly alcohols, ketones, aldehydes and esters in C2-C11.

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P 22. PREDICTION OF FRESHNESS QUALITY OF HADDOCK FILLETS USING ELECTRONIC NOSE, TEXTURE AND TVN MEASUREMENTS

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Abstract

Changes of various properties related to freshness quality of haddock stored in ice were monitored for up to 15 days using traditional methods (sensory analysis, TMA/TVN and microbial counts) and novel instrumental techniques (electronic nose, conductivity measurements, FIGD (flow injection gas diffusion) to measure TMA and TVN and texture analyser. The aim of the studies was to develop models based on different instrumental techniques to predict the sensory freshness score of haddock fillets. Storage studies were done on haddock from different catching seasons and different fishing gear was used. Fish was stored whole in ice and as fillets in styrofoam boxes at 0-2°C. The results show that seasonal variation influences the spoilage rate of haddock and fillets spoil more rapidly than the whole fish as expected. Partial least squares regression models (PLS) based on data from instrumental measurements show that these can give more reliable prediction of the freshness sensory score of haddock than traditional microbial counts and information about storage days in ice.

Introduction

The storage life of fresh fish in ice is short and biological and seasonal variation, catching techniques and handling, influence the spoilage rate and the resulting quality. Raw material is often labelled with days from catch, however because of the effect of various extrinsic and intrinsic factors, the information about days from catch is not always adequate to determine the quality or freshness of the raw material. When good manufacturing practices (GMP) are operated, particularly in respect of temperature control, the information on storage days may be a good indication of the overall quality. Electronic commerce in fish auctions is becoming more widespread, but because of the fact that batches are often mixed in traditional practices it is foreseen that information about the quality grading of the raw material will become more important. Therefore, the commercial partners in the fishery chain need to have access to objective and reliable measurements to verify the freshness quality of the products.

Sensory evaluation of fish fillets is more difficult than evaluation of the whole fish Spoilage signs of the fillets are development of spoilage odor, discoloration and decreased firmness of the flesh. Various instrumental techniques to detect these changes have been developed (Olafsdóttir and others, 1997a) but their implementation in the fish industry has been slow.

Material and Methods

Three storage studies were done on haddock caught at different seasons in spring and autumn and different catching methods were used (longline and Danish seine). The fish was stored whole in ice during the first two experiments, but in the last one the fish was stored as fillets in styrofoam boxes in cold storage (0-2°C). Measurements were done on days 1, 4, 6, 8,11,13 and 15 from catch (Tryggvadóttir and Olafsdóttir, 2000)

Texture measurements were done using a Stable Micro Systems (TA.XT2i) texture analyser to perform TPA test (Texture Profile Analysis) and firmness test (puncture test) .

Electronic nose measurements were done using the "FreshSense" instrument developed by IFL and Bodvaki (Iceland). The instrument is based on static headspace sampling using glass container (5,2L) at room temperature and electrochemical gas sensors(Dräger, Germany: CO, H_2S and SO_2; City Technology, Britain: NH_3). The measurement techniques has been decribed earlier by Olafsdóttir and others, (1997b; 2000)

Determination of TVB and TMA using Flow Injection/ Gas Diffusion (FIGD) analysis according to Capillas & Horner (1999).

Sensory analysis was performed by 10 - 12 trained members of the IFL sensory panel. The Torry scheme was used for cooked fish (Shewan, 1953). Quality grading of fillets was done according to Emilía Martinsdóttir (1995)

Microbial analysis Total viable counts (TVC) were assessed on modified Long & Hammer's medium (Van Spreekens, 1974).

Results and Discussions

Sensory analysis of the fillets using evaluation of cooked fillets according to the Torry scheme showed that the spoilage pattern was different in the three studies. Fillets spoil faster than whole fish as expected and seasonal variation influenced the spoilage rate. Recently spawned fish stored as whole fish in ice from the spring had shorter shelf life (9-10 days) than whole fish stored in ice fish from the autumn season (14-15 days) and fillets stored at 0-2°C spoil faster than whole fish (8-9 days).

The results of the instrumental techniques were in agreement with sensory analysis.

The texture measurements on haddock from the two seasons show that measurements from the spring show generally higher values than the measurements from the autumn experiment. Fish stored as fillets had slightly higher values for hardness (TPA) and firmness (puncture test) than the fillets stored as whole fish.

The electronic nose measurements showed that the response of the sensors were highest for the fish stored as fillets indicating the most rapid spoilage for the fillets The results from the storage study in the spring indicated higher spoilage rate for recently spawned fish than for the fish from the autumn season. The response of the CO sensor correlates well with the total viable counts wheras the NH_3 sensor correlates well with TVN measurements. The responses of the NH_3 and SO_2 sensors detecting microbially produced amines and sulfur compounds, respectively, start to increase at a similar time as end of shelf life is reached in all experiments.

Conclusions

Partial least squares regression models (PLS) based on data from instrumental measurements show that these can give more reliable prediction of the freshness sensory score of haddock than traditional microbial counts and information about storage days in ice.

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P 23. DETECTION OF VOLATILE COMPOUNDS BY AN ELECTRONIC NOSE TO MONITOR FRESHNESS OF HADDOCK STORED IN ICE

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Abstract

The odor of fish is one of the most important quality attributes to determine the freshness quality. An electronic nose (FreshSense) based on electrochemical gas sensors has the potential to be applied for quality monitoring in the fish industry by measuring rapidly the volatile compounds contributing to the spoilage odour of fish. The aim of the study was to identify and determine the level of the most abundant volatile compounds produced in haddock fillets during storage by analysing the headspace volatiles by gas chromatography and comparing the results to electronic nose analysis. The results show that the compounds present in the highest concentration during storage are alcohols, carbonyls, esters, amines and sulfur compounds. The overall trend in the responses of the sensors is comparable to the sum of peak areas of spoilage indicator compounds detected by GC. The development of spoilage odor can be rationalised based on the increasing concentrations of spoilage indicator compounds.

Introduction

The rapid detection of the quality of food has been one of the main application areas for the newly developed electronic nose technique. An electronic nose FreshSense based on electrochemical gas sensors (CO, SO_2 and NH_3) has been used in our laboratory for freshness monitoring of various species of fish i.e. haddock, capelin, redfish and cod (Ólafsdóttir *and others*, 1997a,b;1998; 2000; 2002; DiNatale and others 2001). A similar trend in the development of volatile compounds has been observed. For further development of the electronic nose, information about sensitivities of sensors towards key indicator compounds giving information about quality is needed.

Characteristic odor of fish changes because of the degradation of the tissue and the development of the characteristic spoilage odors of fish have been associated with varying levels of different volatile compounds present in the headspace of fish during storage (Lindsay and others, 1986; Josephson and others, 1986). Microbially formed degradation compounds are present in high concentrations in the headspace of ice stored fish while the compounds contributing to the fresh fish odor and oxidation odors are present in much lower concentrations (Olafsdóttir and Fleurence, 1997).

Materials and Methods

Sample preparation: Haddock fillets were stored in styrofoam boxes in cold storage (0-2°C) for 3, 7, 10 and 14 days.

Gas chromatography analysis: A TENAX tube and an air pump were used to collect the headspace volatiles Volatile compounds were separated on a fused silica capillary column, DB-5ms (J&W Scientific, Folsom, CA) using helium as a carrier gas. GC/MS measurements were performed on a HP G1800C GCD (Hewlett-Packard, Palo Alto, CA). Quantities were estimated based on peak areas.

Electronic nose measurements were done using the "FreshSense" electronic nose developed by IFL and Bodvaki (Iceland). The instrument is based on electrochemical gas sensors (Dräger, Germany: CO, H_2S and SO_2; City Technology, Britain: NH_3). (Olafsdóttir *and others* (1997; 2000).

Sensory analysis of fillets was performed by two trained panellist evaluating the odor according to sensory schemes described by Martinsdottir (1995) and Elliot (1947).

Results and Discussion

Increasing concentrations of spoilage indicator compounds with storage time result in the development of characteristic odors and simultaneously increased responses of the electronic nose sensors are observed.

Table 1. Main classes of headspace volatiles of haddock fillets during storage at 0°C collected by an air pump on a Tenax trap followed by thermal desorption, separation and identification by GC-MS, including odor descriptions and comments on characteristic responses of the electronic nose.

Spoilage compounds identified in the headspace		**Description of odours**
		The odor of the fillet is **FRESH** and **FLAT** in the beginning of storage. Low responses of the sensors are observed on day 3.
Alcohols	**Aldehydes**	
ethanol	acetaldehyde	The first spoilage odors of the fillets are **SWEET** like odors that are contributed by **alcohols** and **carbonyls** that give sweet, solvent like and oxidized-like odors in Increasing response of CO sensor is observed on day 7
2-methyl-1-propanol	3-methyl-butanal	
1-penten-3-ol	hexanal	
3-methyl-1-butanol	heptanal	
2-methyl-1-butanol	nonanal	
2,3-butandiol	decanal	
Ketones		
2,3-butandione		
3-pentanone		
3-hydroxy-2-butanone		
Amines		
TMA		The **amines** contribute to salted fish or stock fish odor and **STALE** odors develop. Increased NH_3 sensor response is observed on day 10.
Esters	**Sulfur compounds**	
ethyl acetate	dimethyl sulfide	The **esters** analyzed in high levels on day 14 have characteristic fruity odors. When these sweet and fruity odors are mixed with the foul smell of the **sulfur** compounds and stockfish- like character of the amines the odor of the fillet becomes **PUTRID.** The response of the SO_2 sensor has increased on day 14.
propanoicacid-2-methyl,ethylester	dimethyl disulfide	
acetic acid, 2-methylpropyl ester	dimehtyl trisulfide	
butanoic acid, ethyl ester		
2-butenoic acid, ethyl ester		
butanoic acid, 2-methyl, ethylester		
butanoic acid, 3-methyl, ethylester		
hexanoic acid, ethyl ester		

Table 1 shows the main classes and identities of compounds present in the highest concentration in the headspace of haddock fillets during storage at 0°C and the spoilage odor development.

Figure 1 shows the responses of the electronic nose sensors compared to the sum of the area of peaks in the gas chromatogram for signals corresponding to each of the main classes of compounds detected during storage of haddock. Earlier studies have shown that the electrochemical gas sensors (CO, SO_2 and NH_3) in the electronic nose "FreshSense" can detect selected standards which are representative of the main classes of compounds present in the headspace of ice stored fish (Olafsdottir, 2002) However, ketones were not detected and have therefore not been included in the graph in Figure 1.

A similar trend of the sensor responses and the concentration of volatiles analysed in the headspace of the haddock fillets during storage is evident. The CO sensor is sensitive to the alcohols, aldehydes and esters, and these contribute to the high response of that sensor. The NH_3 sensor is sensitive to amines, mainly TMA and ammonia and the SO_2 sensor detects volatile sulfides.

The TENAX technique does not detect the very volatile small molecules like ammonia, hydrogen sulphide, methyl mercaptan, and ethanol that are also known to be present in abundance in the headspace of spoiled fish. The electrochemical sensors can however detect these compounds and therefore the slopes and shapes of the curves are slightly different.

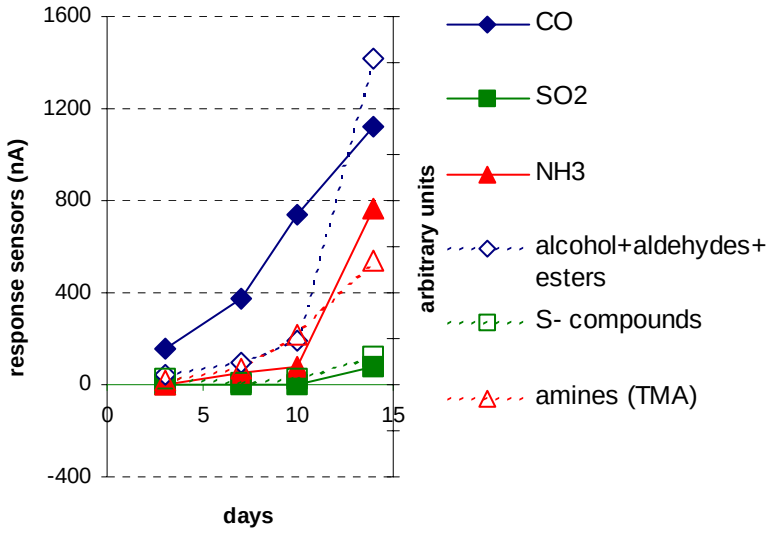

Figure 2 Sum of the peak areas of compounds representing the three different classes of compounds detected by GC in the headspace of haddock fillets during storage in ice and responses of the CO, SO_2, and NH_3 sensors (modified from Olafsdóttir, 2003)**.**

Conclusions

Knowledge about the composition of the headspace of different fish products during storage is useful to guide the development of electronic noses for quality monitoring of fish products. Ethanol, 3-methyl-1-butanol, 2-methyl-1-propanol, 3-hydroxy-2-butanone, ethyl acetate and butanoic acid ethyl ester were the most abundant volatiles in the headspace. Similar volatile compounds were found in cold smoked salmon during refrigerated storage (Joffraud and others, 2001). This is expexted since similar profiles of microflora emerge in different food products under the same conditions despite heterogenity in the outset (Lone and others, 2002). A similar set of sensors can therefore be used for freshness evaluation of a variety of fish species and other muscle food that are processed in a different way but stored at refrigeration temperatures.

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P 24. RAMAN SPECTROSCOPIC STUDY OF STRUCTURAL CHANGES IN HAKE (*MERLUCCIUS MERLUCCIUS* L.) MUSCLE PROTEINS DURING FROZEN STORAGE

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Abstract

This work examines changes in the structure and functionality of hake muscle proteins at frozen storage temperatures known to render very different practical storage lives (–10ºC and –30ºC). Apparent viscosity and dimethylamine (DMA) formation showed drastic temperature-related differences during storage. Raman spectroscopy revealed the occurrence of some structural changes involving secondary and tertiary protein structures. The changes in secondary structure were quantified, showing an increase of β-sheet at the expense of α-helix structure. The νC-H stretching band near 2935 cm^{-1} increased in intensity, indicating denaturation of the muscle proteins through the exposure of aliphatic hydrophobic groups to the solvent. These structural changes were more pronounced at –10ºC but occurred at both storage temperatures, whereas changes in apparent viscosity and DMA only occurred in storage at –10ºC. The possible utility of these structural changes for quality assessment is discussed.

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P 25. ADDED WATER IN SCALLOP ADDUCTOR MUSCLES METHODS TO CHECK UNFAIR PRACTICE

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Abstract

Demand for scallops increased mainly in United States, European Union and Japan. Markets stimulate scallop international trade, and if scallop products are characterised by high retail price, they still suffer from quality disparities. During the last decade, added water in scallop adductor muscles have been established by authorities. Those practices disturb scallop trade and depreciate product value.

Scallops need large quantities of water to achieve good washing and hygienic practice. However, the scallop adductor muscle absorb water during preparation and processing. This ability of the scallop muscle to soak-up water is a powerful economic incentive to swindle seafood buyers. In the absence of scallop international standard, the present study reviews and compare methods to detect unfair practices.

In conclusion, depending upon the manner by which scallop muscles are handled, water may be added to the meats by their exposure to fresh water. Few methods look suitable to check fraud from added water by traditional washing operations and biological variations.

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P 26. HYBRID STRIPED BASS - A QIM SCHEME FOR A MAJORU.S. AQUACULTURE SPECIES

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Abstract

Hybrid striped bass (HSB), a cross between striped bass *(Morone saxatilis*) and white bass *(M.chrysop*s), is rapidly becoming a major product of U. S. aquaculture. The HSB industry has been expanding at an annual rate of 7 percent in the U.S. In 2000, HSB was ranked fifth in volume (5,097 mt) and value (US$29.5 million) of all food fish grown. In North Carolina, 22 HSB growers produced 1,091 mt of fish valued at US$5.7 million in 2002. Farm-raised HSB is very popular in the U.S. because of its favorable sensory characteristics and long shelf life. The flesh is white with a mild odor and flavor and a firm and juicy texture. Shelf life of whole fish in ice is 14 days and they are commonly marketed between 0.45-1.14 kg in weight. This poster presents results in development of a Quality Index Method (QIM) scheme for hybrid striped bass. U.S. growers and buyers can use the QIM scheme to make quantitative sensory assessments of fish quality. The QIM parameters used in the HSB scheme are skin color/appearance, discoloration, odor and texture of the whole fish, appearance and shape of the eyes and color/appearance, mucus and odor of the gills. Each QIM parameter is given a demerit point (0-2) and the sum of the points is the quality index (0-16).

Introduction

Although early attempts to culture striped bass were made in the 1970s, it was not until the mid-1980s when Kent SeaTech Corp. (formerly Aquatic Systems, Inc.) produced large quantities of hybrids for the food fish market. Kent SeaTech successfully developed high-density tank culture using geothermal groundwater in southern California. Soon after, extensive pond culture methods were developed in North Carolina and South Carolina. The early success of pond culture can be attributed to research funding by the National Sea Grant Program and South Carolina Marine Resources Department. Numerous tank and pond culture facilities were developed in late 1990s in North Carolina and other southeastern states. The hybrid striped bass market has remained strong with rapid growth in development of live domestic markets and fingerling trade with Asian countries. Current restraints on the U.S. HSB industry are adequate supply of fingerlings, domestication of brood stocks and consistency in the appearance and fresh shelf life of whole fish. This study was conducted as part of a North Carolina fishery resource grant investigating the effectiveness of sodium chlorite for extending the fresh shelf-life of whole HSB. U.S. growers and buyers expressed an interest in a quality scheme to make quantitative sensory assessments of fish quality. The objectives of the study were to develop a QIM scheme for farm-raised hybrid striped bass and to introduce the QIM concept for assessing fish quality to the North Carolina fish and aquaculture industries.

Materials and Methods

Whole fish (Figure 1) were obtained from two local farms (Pure Water Aquaculture, Creswell, NC and White Rock Fish Farm, Vanceboro, NC). The fish were dipped from ponds by nets into chill tanks to render them senseless before packing on ice for transport. Whole fish were packed in ice and held in a walk-in cooler at <2 o C. Fish were examined to describe all detectable quality parameters during storage in ice. The descriptions served as a basis for development of a QIM scheme. At least 9 fish were used from each of two sample dates in this preliminary study. The sensory analysis of cooked fish was performed near the end point of shelf life to judge acceptability in terms of odor, taste and texture. Photographs of each stage in sensory evaluation were taken. Photographs are used to illustrate quality attributes when introducing the QIM concept for assessing fish quality to the North Carolina fish and aquaculture industry members.

Figure 1

Results and Discussion

The QIM scheme developed for HSB is shown in Figure 2. Parameters used in the HSB scheme are skin color/appearance, discoloration, odor and texture of the whole fish, appearance and shape of the eyes and color/appearance, mucus and odor of the gills. Each QIM parameter is given a demerit point (0-2) and the sum of the points is the quality index (0-16). The shelf life of fresh HSB was determined to be 10 days in ice for high quality and 14 days in ice for acceptable quality. Comments received from North Carolina industry members were very favorable. Industry expressed interest in learning more about the QIM scheme and offered additional quality parameters for inclusion in future studies. The need to correlate raw fish with cooked fish quality parameters was of great interest to fish growers concerned about shelf life extension for their products.

Quality Parameters		Descriptions	Points
Whole fish	Skin color/appearance	Pearl-shiny, iridescent pigmentation all over	0
		Less pearl-shiny, yellowish, stripes still distinct	1
	Discoloration (red spots, bruising)	Slight to none	0
		Minor (5 - 10%)	1
		Severe (10 - 25%)	2
	Odor	Neutral, pond, fresh fish, seaweed	0
		Melon, cucumber, green grass	1
		Cardboard, fishy, putrid, rotten	2
Eyes	Pupil	Black, clear, bright, iridescent	0
		Dark gray, mat, dull	1
		Milky, cloudy, hazy, light gray	2
	Shape	Convex, bulging	0
		Flat	1
		Concave, sunken	2
Gills	Color/appearance	Bright red, red, burgundy, uniform in color	0
		Pale red, pink, light brown	1
		Brown, dull, non-uniform in color	2
	Mucus	Transparent, clear, none	0
		Milky, clotted	1
	Odor	Pond, fresh fish, fresh rain	0
		Melon, cucumber	1
		Musty, fishy, putrid, rotten	2
Texture		In rigor	0
		Firm, resilient, finger mark disappears immediately	1
		Soft, finger mark still persists after 3 seconds	2
Quality Index (total score)			0-16

Figure 2

Future Work

Additional trials are planned including involving more fish farms and sampling at different times of the year. Work will involve checking linearity of QIM versus time on ice and determining the correlation for QIM of whole fish to Quality Descriptive Aanalysis (QDA) of cooked fillets**.** Plans are to involve several industry growers in the development process and conducting industry training workshops for use of the QIM scheme on an industry-wide basis.

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P 27. PROTEIN CONTENT IN BRINE FROM SALTED HERRING MEASURED BY NEAR-INFRARED SPECTROSCOPY A POSSIBLE FAST INDICATOR OF RIPENING

(Not to be quoted without prior reference to the author)

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Abstract

Traditionally barrel-salted herring is stored cold for several months where a ripening process takes place. During the storage period myofibrillar proteins as well as small peptides and free amino acids are extracted from the herring muscle into the brine. Multivariate analysis of data from earlier ripening studies shows a good correlation between the increase in nitrogenous compounds in brine and texture change of the fillet, which is an important quality parameter of salted herring.

The objective of this study is to investigate if near-infrared (NIR) spectroscopy can be used as a fast and non-invasive method to measure protein content in brine of salted herring during storage. 20-25 ml brine was sampled regularly from each of a number of barrels with salted herring during a period of six months. Protein content was measured by Kjeldahl analysis. NIR spectra were measured with an infraprover, II fourier transform spectrometer using a cuvette with a light path of 2 mm. Kjeldahl analysis shows that nitrogenous compounds in brine, calculated as protein (total nitrogen x 6.25), increases up to 5-6% during the storage period. Multivariate analysis shows good correlation between NIR spectra and the increase in protein content. The results indicate that NIR spectroscopy could be a potential fast method for determining protein in brine and could therefore be a possible fast method to obtain information of the ripening process.

Introduction

Salted herring is an important product for the pelagic fish industry in the Nordic countries. The production process is bound by tradition and is based on human knowledge and experience. Understanding of the biochemical and chemical processes governing the ripening process is still limited and great interest lies in getting a better understanding of the unique taste and texture development that happens during the months long ripening period. In a previous study by Nielsen and co-workers (1999) data from ripening experiments carried out at three Nordic Fisheries research institutes were collected and analysed in order to investigate if further information about the salting and ripening of herring could be derived by combining data from different ripening experiments. The results showed correlation between a number of the basic chemical analyses and important sensory parameters like texture and ripened taste. The results indicated that the amount of protein in brine might be used as indicator variables since it correlated with a number of the chemical and sensory parameters related to ripening including softness. It is shown that a major part of the protein is extracted into the brine during storage is myofibrillar proteins (Nielsen 1995). It was shown that the protein content in brine correlates with trichloroacetic acid-soluble nitrogen in muscles, which expresses the degree of protein degradation during ripening of salted herring (Nielsen et al. 1999; Bro et al. 2002). These results is one of the motivations for the present study as sampling of brine is more accessible and representative than sampling of a whole fish, where the in homogeneity of the fish has to be taken into account. The change in protein concentration in brine instead of the protein degradation in the herring may therefore be an indicator variable for the ripening of herring. Near-infrared (NIR) spectroscopy is well suited for determining the major components of foods such as water, fat, and protein and is non-destructive, fast and easy to implement. The objective of the present study is to test if NIR spectroscopy can be used to determine the protein content in brine from traditionally barrel-salted herring. Multivariate data analysis and prediction modelling between NIR spectroscopy and the protein concentration is used as evaluation tools to study the relation between the spectroscopic measurements and the protein content.

Materials and Methods

Barrels with salted herring were prepared as followed: 100 kg of whole headed herring was mixed with 10 kg of salt. After one day the barrels were filled with saturated brine and stored at 0-5°C. Two storage experiments with eight barrels and four barrels respectively were carried out. 20-25 ml of brine was taken for analysis from each barrel at each sampling during a period of six months. The brine was centrifuged at 10000g for 20 min at 5°C to remove tissue parts and insoluble matter and kept at –20°C until analyses was carried out.

Protein content: Protein content of the brine was determined by the Kjeldahl method (total n x 6.25).

NIR measurements: The NIR spectra were measured with an infraprover, II Fourier transform spectrometer (Bran and Luebbe, Germany) using a cuvette with a light path of 2 mm (Hellma fluorescence cell with four windows). Spectral range of the NIR spectroscopy from 10000 to 4000 cm^{-1} (1000 to 2500 nm) was used.

Multivariate data analysis: Multivariate calibration on the NIR spectra was performed using partial least squares regression (PLS) and protein concentration as the dependent variable. The PLS model was validated using leave-one-out cross validation

Results

In figure 1, a result from the study of Nielsen et al. (1999) is shown, where a scatter plot indicate a correlation (r is 0.84) between the sensory attribute softness of salted herring and the protein content in brine. Data is from ripening experiments carried out at the Icelandic Fisheries Laboratories, Norconserv (Stavanger) and Danish Institute for Fisheries Research.

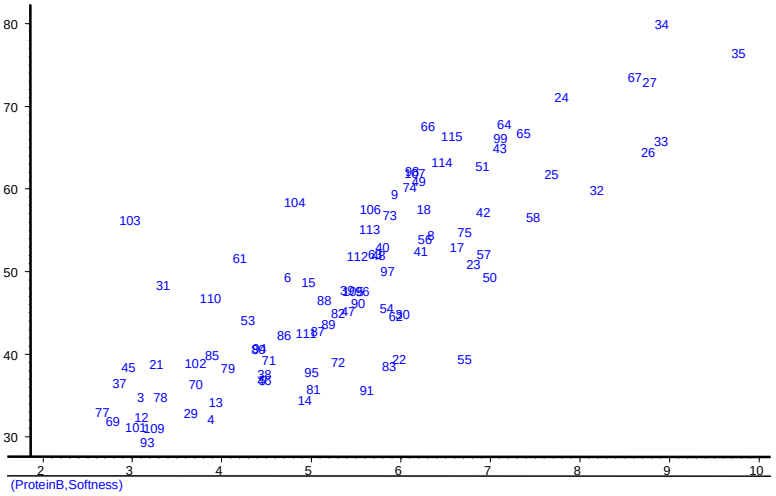

Figure 1. Scatter plot of protein in brine versus softness for salted herring. Correlation is 0.82 (from Nielsen et al. 1999).

In the present study a PLS regression performed on the entire NIR spectra of brine samples gave a five factor model describing 78.4% of the y-variance and 90.5% of the x-variance, r is 0.87 and the *rmsecv* is 0.34%. In figure 2 the predictive performance of the PLS regression model is illustrated with predicted protein (cross-validated) content versus the actual protein content.

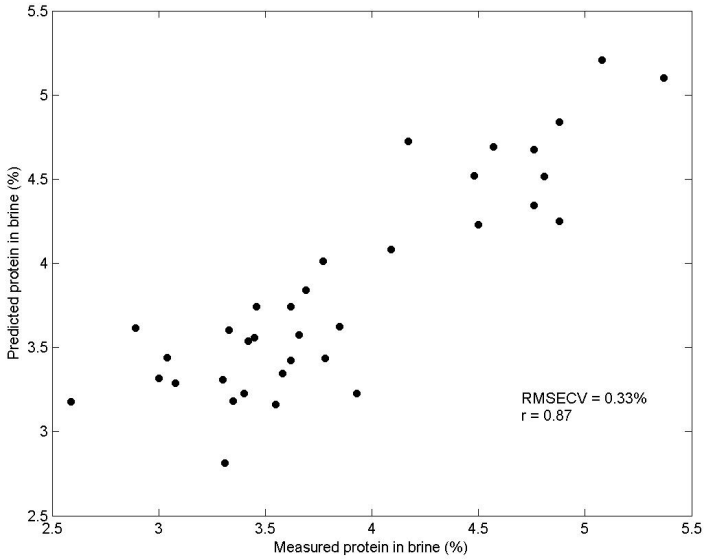

Figure 2: A five factor cross-validated PLS regression model predicting protein in brine from NIR data.

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P 28. A HANDHELD TDR-SYSTEM FOR THE DETERMINATION OF THE QUALITY OF SEAFOOD

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Abstract

Time-Domain-Reflectometry (TDR) is a technique which has been applied for the determination of the quality of seafood [1],[2]. In many cases, however, the technical and especially the financial effort is too high for making time-domain measurements in a sufficient frequency-range (use of a RF-Sampling-Oscilloscope).

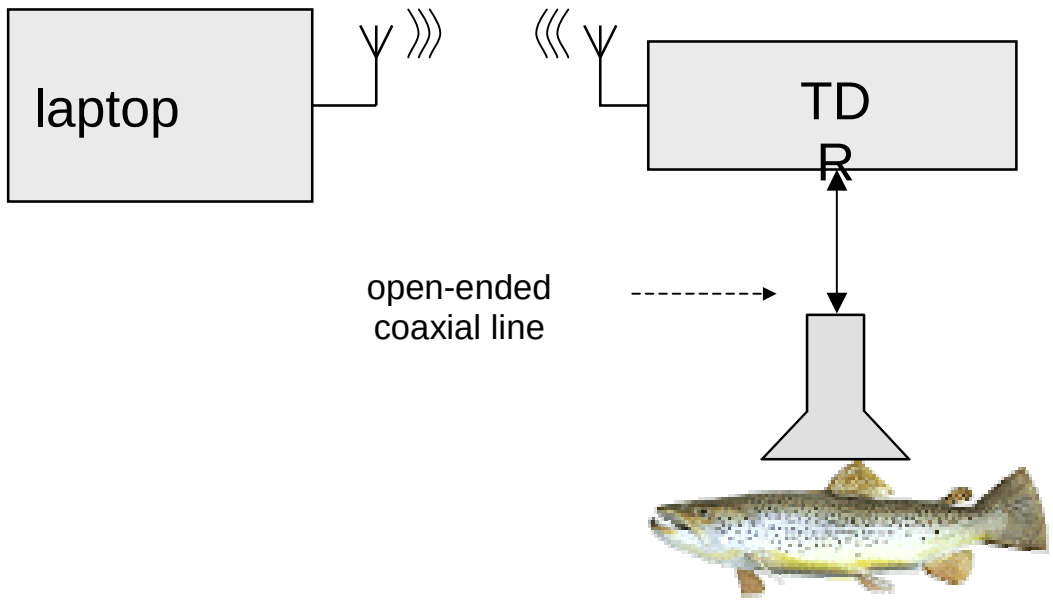
Due to the progress of mobile communication systems of the 2nd and 3rd generation (GSM, PCS, W-CDMA) the design of low-cost integrated circuits in the microwave frequency-range has been accelerated. This increasing integration of RF-systems has led to a wide range of commercial components available for constructing a handheld TDR-system with a low power-consumption, that fulfills the practical measurement requirements imposed on quality sensing systems.

This poster is about a TDR-prototype with a system-risetime of t_r < 100 ps, equivalent to a maximum frequency of approximately $f_{max} \approx 6$ GHz. The time-base resolution t_s=10 ps of the device is achieved by using the sequential-sampling technique.

For easy handling the measurement and display software is implemented on a Laptop/PC with graphical-user-interface (GUI). The TDR-prototype and the computer are linked by a wireless connection to ensure mobility.

By using multivariate calibration techniques, discriminant analysis and artificial neural networks, the instrument extracts a number of variables of interest e.g. time of storage, temperature of storage, QIM and other sensory scores from measured microwave signals.

Materials and Methods



Using Time-Domain-Reflectometry (TDR) means, that a electromagnetic step impulse is generated and applied to the material under test. The required step signal can easily be generated, utilising a radio frequency integrated circuit (RFIC), for example a frequency prescaler IC. The seafood is brought into contact with an applicator, which is realised as an open-ended coaxial line with an extended ground plain. The step signal is reflected at the surface of the seafood and carries information about the material. Subsequently, the reflection is acquired in a sampling gate and is analog-to-digital converted to be stored in a microcontroller. Finally the data is transferred into a computer, to display the signal and for further high-level data processing. The prototype instrument is realised in microstrip technology and SMD-technology, which ensures an inexpensive hardware setup.

The methodology has been investigated in long-range series of measurements with Cod, Hake, Salmon and Bass [3]. Combining TDR technology and multivariate calibration techniques, respectively artificial neural networks leads to very promising results concerning the prediction of time of storage, the storage temperature and QIM.

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P 29. DETECTION OF PARASITIC ROUNDWORM IN ATLANTIC COD (Gadus morhua)

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Abstract

The increasing quantity of parasitic infection in cod (*Gadus morhua*) observed in later years has brought attention to several issues regarding this problem. Parasites in fish products tend to spoil consumers appetite when present. Recent reports questioning the safety of eating cod infected with parasites might lower the consumer acceptability toward seafood. Today, parasites are detected and removed manually. It is reported an average efficiency about 75% under commercial conditions. In this study, we have focused on biochemical differences between cod muscle and the prevalent anisakine nematode species (*Anisakis simplex* and *Pseudoterranova decipiens)* infecting Atlantic cod. Using reversed phase HPLC equipped with a photodiode-array detector, substances absorbing in the range 300-600 nm were identified in extracts from parasite material. These substances were not detected using the equivalent procedure in extracts from cod tissue. Significant biochemical differences between cod muscle and parasite material has so far been validated, but further testing is required before a suitable method for detecting parasites in fish fillets can be presented.

Introduction

Infection of parasitic nematodes in Atlantic cod, and the difficulties in detecting these parasites during processing of fish fillet reduces the quality and increase the cost of fish brought to the market. Two species of anisakine nematodes are prevalent from infected Atlantic cod, *Anisakis simplex* and *Pseudoterranova decipiens.* These parasites infect the fish in its larval stage, and their final hosts are seals and whales, where the development of their reproductive stages occurs.

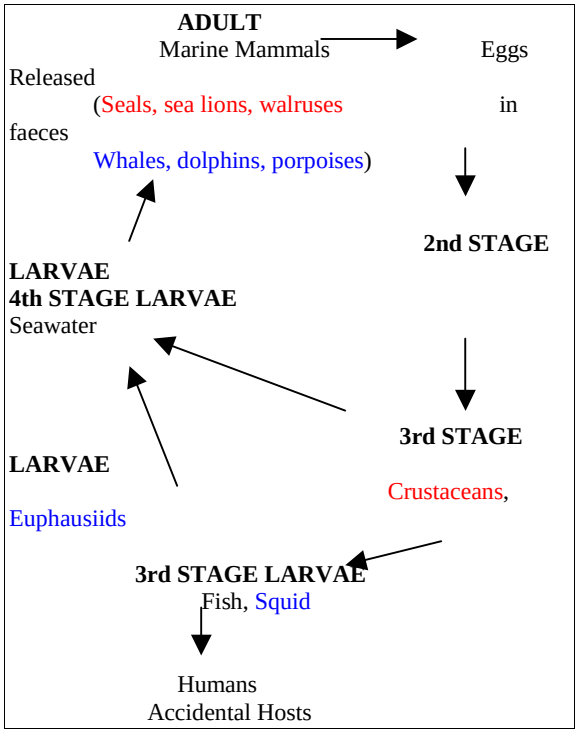

Figure 1 Life cycles of *Anisakis simplex* and *Pseudoterranova decipiens*.

The fact that humans can act as accidental hosts for these parasitic nematodes was first recognised in Holland in the 1960s. This condition, called Anisakiasis, is most commonly acquired by eating raw or inadequately cooked fish, and proper processing of the fish before consumption should eliminate any danger of infection. Although

these parasites pose but a small threat to humans, consumers generally reject the product if parasite infection is present.

In this study we aim to contribute to the development of technology that can replace manual operations in fillet processing. The main objective is to identify substances found in parasites but not in fish muscle. We are particularly interested in compounds that yield specific optical or electromagnetic signals that can act as signatures in a method for detecting parasitic infections in fish flesh.

Results

Using reversed phase HPLC equipped with a photodiode-array detector, substances absorbing in the range 300-600 nm were identified in extracts from parasite material (Figure 2 and 3). These substances were not detected using the equivalent procedure in extracts from cod tissue (Figure 4).

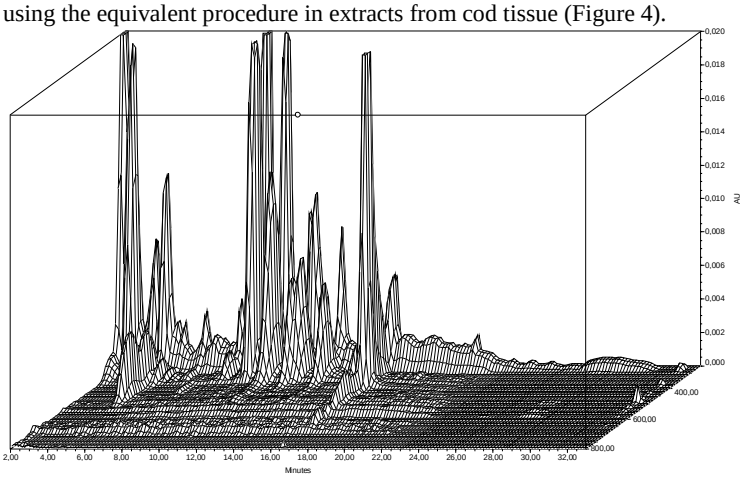

Figure 2 HPLC analysis of *P. decipiens*

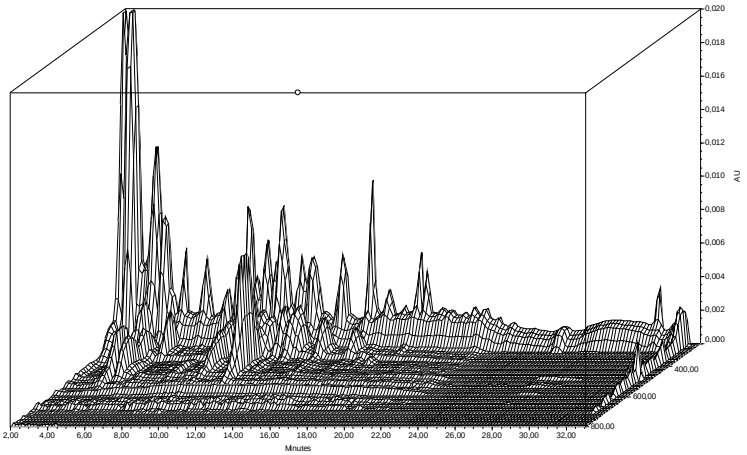

Figure 3 HPLC analysis of *A. simplex*

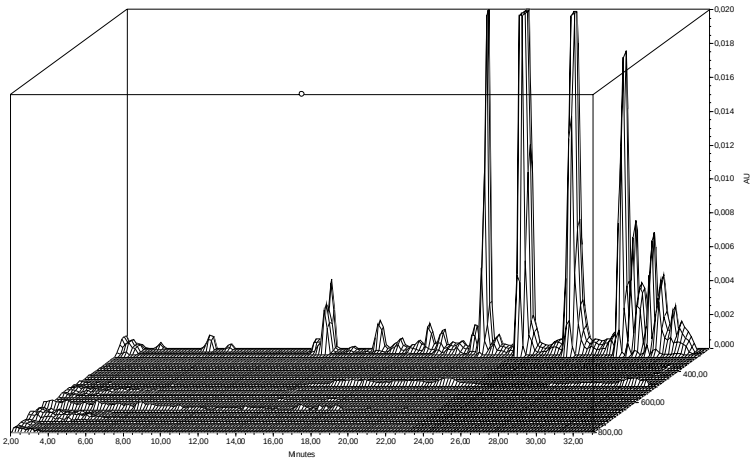

Figure 4 HPLC analysis of cod (*Gadus morhua*)

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P 30. METHODS FOR MEASURING LIPID OXIDATION AND THEIR APPLICATION TO FISHMEAL

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Abstract

Fishmeal manufactured from pelagic fish is a highly nutritious and valuable as feed ingredient. The nutritional benefits of fishmeal are attributed to high content of protein, minerals and n-3 fatty acids originating from unsaturated fish lipids. Fishmeal is particularly susceptible to oxidation, which is further induced by the heating and drying during processing. Lipid oxidation is therefore one of the major degradation processes that takes place in fishmeal. In spite of a variety of methods available to measure lipid oxidation there is no universally recognised method for the determination of the stability of fishmeal. The objective of the present work was to examine methods for measuring lipid oxidation and their application to fishmeal. Methods for assessing lipid oxidation were compared in fishmeal from capelin and herring. Changes during storage of fishmeal were measured as the development of primary, secondary and tertiary lipid oxidation products. The results of this study demonstrated that the methods most suitable for measuring changes of fishmeal with storage time were those that measure advanced lipid oxidation.

Introduction

Fishmeal from small pelagic fish is made from the whole fish and is therefore very nutritious with high content of protein, minerals and n-3 fatty acids. It is generally accepted that n-3 unsaturated fatty acids have nutritional benefits, but they are also more susceptible to oxidation. This is particularly true for fish meal, which is heated and dried during processing. The consequences of lipid oxidation in fish meal can appear as protein damage and reduced nutritional value of the meal (Opstvedt 1975; Pike and others 1990). Lipid oxidation in fish meal has been known to proceed as far as causing heat damage of the meal and even lead to combustion (Ólafsson 1953; Waissbluth and others 1971).

Traditional methods for measuring lipid oxidation have often showed inconsistent results when applied to fish meal, probably due to advanced oxidation of the fish meal lipids. Other more indirect methods have been reported as indicators of lipid oxidation in fish meal. Decline in extractable lipids of fish meal with time has been pointed out as indicator of oxidation (Barlow and Pike 1977), as well as the recoveries of the most polyunsaturated fatty acids (Ackman and Gunnlaugsdottir 1992). These methods have however, not been generally recognised for determination of the stability of fishmeal. The present investigation was undertaken to compare different methods from the food and frying oils sectors for measuring lipid oxidation in fishmeal.

Materials and Methods

The fish meal was obtained from SR-mjöl hf fish meal factory in Seyðisfjörður. The samples were stored in closed plastic bins (2 L) in the dark at 10 °C. Selected methods for measuring lipid oxidation were tested on capelin meal (C) and herring meal (H) from different seasons (no. of month). One sample CE7 contained ethoxyquin (500 ppm) as antioxidant and was made from the same lot as C7. The fish meal was measured for water (ISO, 1983) and fat content (AOCS 1998) using petroleum ether (Bp. 30-40 °C) for fat extraction. Further analyses of lipids were carried out after extraction with chloroform/ methanol (Bligh and Dyer 1959). The lipids were measured for refractive index (RI) and polar components according to Official Methods (AOAC 1990) as well as conjugated dienes (CD) and conjugated trienes (CT) (IUPAC, 1987). Fluorescence Products (FP) of lipids in chloroform were measured as total fluorescent lipid oxidation products (peak area units per mg of lipid) with an excitation maximum at 355 nm and emission maximum at 437 nm in a luminescence spectrometer (LS 50B, Perkin Elmer Instruments, Beaconsfield, Bucks, UK). Keeping qualities of fish meal were measured by the electronic nose (EN) "FreshSense" (Element Sensor Systems, Sauðárkrókur, Iceland) to monitor the headspace of the meal (Ólafsdóttir and others 1997). The induction period (IP) of fish meal was measured on 50 g samples under 5 bars oxygen pressure in Oxipres apparatus (Mikrolab Aarhus A/S, Højbjerg, Denmark). The pressure was recorded at 90 °C and the IP determined graphically form the pressure drop resulting from oxygen consumption of the sample. Brown pigments (BP) were measured as acetic acid soluble color (Maruf and others 1990). The color of the meal was measured by a Minolta CR-300 Chroma meter (Minolta Camera Co., Ltd., Osaka, Japan). Results were given as L*, a* and b* values and as total change in initial color: $\Delta E^*ab = \Sigma((\Delta L^*)_2 + (\Delta a^*)_2 + (\Delta b^*)_2)$.

Results

The induction period (IP) was found from the Oxipres readings when the pressure declined as a result of oxygen uptake of the lipids (Figure 1). The Oxipres readings as well as the IP demonstrated that the herring meal

was less stable than the capelin meal, besides that the antioxidant addition in capelin meal sample CE7 was more stable than the corresponding capelin meal without antioxidant C7.

The results for water and lipid content as well as quality parameters measured in the fish meal during storage at 10°C are compiled in Table 1. The capelin meal with antioxidant addition (CE7) was not quite homogenous in lipid and water content compared to the sample from the same lot without antioxidant (C7), but some parameters changed to such intent that they may be regarded as indicative for oxidation in the meal. A 32% decrease in extractable lipids was observed during 4 months storage in the herring meal H6, compared to a 4% decrease in the capelin meal C11 and a 2% decrease in C7. Antioxidant addition did reduce the lipid decrease to 0.6% in CE7. The measurements that changed most during storage in samples H6 and C11 were besides the lipid content, brown pigment (BP), fluorescence products (FP), change in color (ΔEab*) and CO measured with the electronic nose, The induction period (IP) was also lowest in these two samples. The antioxidant addition in C7 had most effect on the change in BP, ΔEab* and the initial CO but not on the change in CO during storage, the IP was also much higher in the antioxidant treated meal.

The best correlation between measurements were seen between the change in extractable lipids and ΔEab* (r = 0.96), as well as in the change in BP (r = 0.92), and a negative correlation with the induction period (r = -0.86).

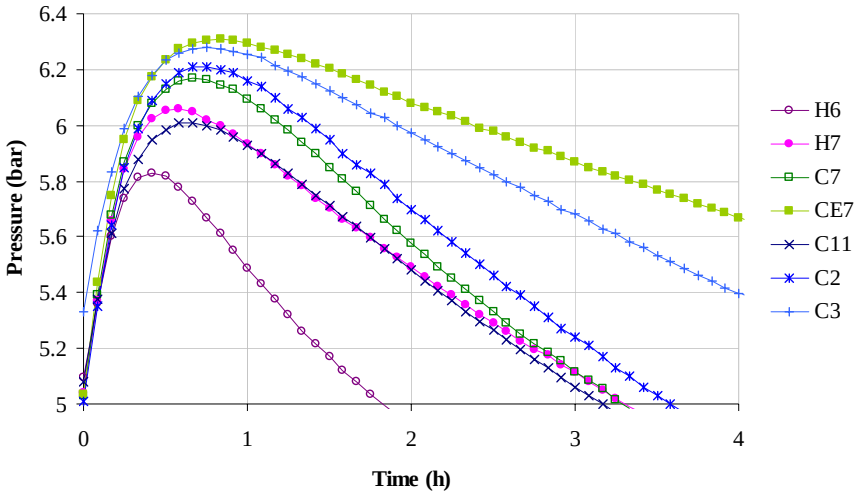

Figure 1 - Oxipres readings of fish meal.
Abbreviations for samples: H = herring meal, C = capelin meal, CE = capelin meal with ethoxyquin (500 ppm), numbers represent no of harvesting month.

Table 1 - Changes in fish meal during storage (10°C).

	Storage time	Water	Lipid	IP	BP	FP	RI	CD	CT	PC	Color				Electronic nose (nA)			
Sample	(mo)	(%)	(%)	(min)	(A/g)	(F/mg)		(A/g)	(A/g)	(%)	L*	a*	b*	ΔE*ab	CO	H2S	SO2	NH3
C2	0	6.61	11.47	57	0.392	179	1.4865	57	14	62	50.57	0.78	11.59		538	157	24	722
	2		11.31		0.572	293	1.4867	76	21	51	49.43	0.62	13.46	2.28	442	210	44	794
	4		11.36		0.567	419	1.4875	91	25	42	50.05	0.79	13.66	2.17	621	195	54	823
C3	0	9.02	8.36	58	0.256	163	1.4870	62	13		46.70	0.17	9.93		207	182	32	644
	2		8.59		0.316	221	1.4860	85	19	58	46.66	0.23	10.59	0.81	525	176	46	846
	4		8.42		0.288	502	1.4873	109	25	40	46.67	0.39	10.74	1.19	566	181	53	815
C7	0	5.63	9.40	54	0.962	234	1.4854	81	20	52	54.12	0.98	15.64		3107	34	30	396
	2		9.32		0.993	298	1.4854	107	26	54	54.77	0.96	16.05	0.79	3636	54	38	524
	4		9.21		1.017	200	1.4872	82	19	52	54.20	1.22	17.00	1.44	3007	57	56	314
CE7	0	6.91	7.77	65	1.165	181	1.4850	81	19	53	52.79	1.41	15.41		309	26	16	872
	2		7.77		1.174	215	1.4857	125	27	55	53.30	1.34	15.55	0.54	283	9	14	752
	4		7.73		1.165	363	1.4866	93	26	56	53.72	1.54	15.72	1.02	321	26	26	587
C11	0	6.75	10.87	52	0.457	270	1.4857	78	19	39	51.54	0.38	11.77		457	348	50	2110
	2		10.67		0.834	433	1.4864	108	30	56	50.55	0.47	14.51	2.92	657	413	83	2175
	4		10.45		0.931	613	1.4864	110	31	55	51.32	0.49	15.05	3.29	688	382	100	1897
H6	0	7.55	8.06	32	1.37	55	1.4820	70	14	45	52.96	4.16	17.87		835	291	53	1171
	2		6.23		2.002	198	1.4841	151	67	54	52.53	4.13	21.77	3.93	1645	182	222	535
	4		5.49		2.212	595	1.4847	183	98	50	51.10	4.72	23.77	6.27	1780	188	265	423
H7	0	6.53	13.14	45	1.199	6	1.4752	112	9	34	55.96	4.38	20.02		1035	237	57	2878
	2		13.02		1.309	73	1.4761	121	14	33	55.40	4.24	21.58	1.67	616	52	66	1934
	4		12.95		1.359	89	1.4762	116	15	33	55.49	4.59	21.84	1.90	596	29	72	1763

Abbreviations H = Herring meal, C = Capelin meal, CE = Capelin meal with Ethoxyquin (500 ppm), IP = Induction Period, BP = Brown Pigments, F = Fluorescence Products, RI = Refractive Index, CD = Conjugated

Dienes, CT = Conjugated Trienes, PC = Polar Compounds, L* = lightness, a* = red, b* = yellow, ΔE*ab = change in initial color.

Conclusions

Capelin meal was more stable towards oxidation than herring meal and antioxidant addition (500 ppm ethoxyquin) in capelin meal demonstrated increased stability.

The methods most suited to measure lipid oxidation in herring and capelin meal were those that measure changes in lipid content, color and brown pigments as well as the induction period obtained from the Oxipres stability test.

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P 31. EFFECT OF FREEZER TEMPERATURE AND STORAGE TIME ON THE SURVIVAL OF SPECIFIC SPOILAGE ORGANISMS IN COD MINCE

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Abstract

This study aimed at assessing the effect of freezer temperature (-20, -24 and -30°C) and storage time (from 1 day up to 52 weeks) on the survival of specific spoilage organisms in inoculated cod mince upon thawing and during chilled storage under modified atmosphere (MA). In these trials, fish mince was prepared from newly caught cod and inoculated with a cocktail of *Photobacterium phosphoreum* and *Shewanella putrefaciens*. Nine treatments were assessed based on 3 contamination levels and 3 freezer storage temperatures. Contamination level was assessed prior to freezing and after 1 day, 4-7-15 and 52 weeks of storage. Pieces of thawing cod mince were thereafter MA-packed, stored at 0.9 ± 0.1°C and their microbiological counts (total psychrophilic counts (TVC), *P. phosphoreum* and H_2S-producers/*S. putrefaciens* counts) followed weekly for 3 weeks. This allowed for a better evaluation of the effect of freezer temperature and storage time on the survival of the bacteria of concern. The results indicate that freezing had little effect on TVC and H_2S-producers/*S. putrefaciens* counts, while a significant reduction of *P. phosphoreum* cells was observed after 1 day of freezer storage at all temperatures and contamination levels tested. The freezer temperature of -20°C was the most drastic treatment for *P. phosphoreum* cells as lower counts were generally observed. Growth delay of *P. phosphoreum* under MAP chilled conditions was prolonged with increasing freezer storage time at all contamination levels. In fact, further freezer storage led to an overall reduction of microbiological counts for all treatments, the greatest effect being observed at -20°C.

Introduction

Recently published work has demonstrated that *Photobacterium phosphoreum* can be inactivated during frozen storage of cod (Guldager et al., 1998; Boknæs et al., 2000). These findings are certainly relevant since this bacterium has been found to produce large amounts of trimethylamine (TMA) in fresh MAP cod, hence being the main spoilage bacterium in this product (Dalgaard et al., 1993). A storage period of only 4 weeks at –20°C inactivated low levels of *P. phosphoreum*. Moreover it was found that inactivation can be more efficient at –20°C than –30°C, depending on the quality of cod (microbiological load) and its origin. In fact it was reported that a higher concentration of TMAO and NaCl in cod muscle can protect the bacterium from being totally inactivated (Boknæs et al., 2001). However, nothing is known about the effect of different freezer temperatures and storage time on the behaviour of this bacterium as well as other spoilage bacteria in cod caught in the Icelandic fishing grounds. This study aimed at assessing the effect of freezer temperature and storage time on the survival of specific spoilage organisms in inoculated cod mince upon thawing and during chilled storage under modified atmosphere. This is part of a national project, funded by The Icelandic Research Council, evaluating the potential use of sea-frozen cod fillets in the preparation of thawed, MA-packed retail products.

Materials and Methods

Preparation, contamination of cod mince – Packing and freezing of samples. Newly caught, gutted cod was shipped to IFL, filleted, skinned, trimmed and minced in a sterile mincer. About 10 kg of mince was divided in 3 parts: 1 part used as the control, while the 2 other parts were contaminated with strains of *Shewanella putrefaciens* and *P. phosphoreum* (3 of each) to reach a level of 10^{2-3}/g and 10^{6-7}/g of mince. The mince was portioned into bags (PET 12/LLDPE 50, 15 x 20 cm, Plastprent Ltd.), 70-105-140 or 210 g, rolled to make a sausage, and 2 rolls put into another bag, then vacuumed to 80% prior to freezing at –20, -24 and –30°C. Samples were stored at each respective temperature for 1 day, 4-7-15 and 52 weeks.

Evaluation of microbiological counts. Samples were assessed prior to freezing and after 1 day, 4-7-15 and 52 weeks of storage at –20, -24 and –30°C. At each sampling time, 1 vacuumed bag (including 2 rolls) was removed from each storage temperature for each contamination level. Duplicate samples were analysed for total psychrophilic counts, using modified Long & Hammer's medium with 1% NaCl (spread-plated LH), as well as for H_2S-producer counts (poured Iron agar with 1% NaCl) and *P. phosphoreum* counts using a conductance method (Dalgaard et al., 1996). Pieces of thawing cod mince (35-40g) were also MA-packed ($CO_2/O_2/N_2$: 60/10/30), stored at 0.9 ± 0.1°C and their microbiological counts followed weekly for 3 weeks.**Results and Discussion**

The results indicate that freezing had little effect on TVC and H_2S-producers/*S. putrefaciens* counts, while a significant ($p<0.05$) reduction of *P. phosphoreum* cells was observed after 1 day of freezer storage at all temperatures and contamination levels tested (Table 1). After 4 weeks of freezer storage, *P. phosphoreum* could not be detected upon thawing of the samples at the lower contamination levels. However MAP chilled storage of

thawed samples for 1 week revealed its presence. The freezer temperature of -20°C was the most drastic treatment for *P. phosphoreum* cells as larger drops in counts were observed at higher contamination level. Similarly to Boknæs et al. (2002), growth delay of *P. phosphoreum* under MAP chilled conditions was prolonged with increasing freezer storage time at all contamination levels due to decreasing cell numbers at thawing. After 15 weeks of freezer storage, the level of *P. phosphoreum* in highly contaminated mince had dropped by ca 2, 4 and 6 log/g with increasing freezer temperature. *P. phosphoreum* could not be recovered after 52 weeks freezer storage and 3 weeks of chilled storage, independently of the contamination level and freezer temperature. In fact, further freezer storage led to an overall reduction of microbiological counts for all treatments, the greatest effect being observed at -20°C. Levels of *S. putrefaciens* were also significantly reduced after 4 weeks of freezer storage, the greatest effect being observed at -20°C, higher contamination level and with further storage. After 52 weeks, levels of *S. putrefaciens* were just at the detection limit in low contaminated mince at all temperatures, similarly to highly contaminated mince stored at -20°C. Interestingly, the decreasing psychrophilic counts observed in highly contaminated mince with prolonged freezer storage time corresponded to the levels of *S. putrefaciens* found at each sampling time, except after 52 weeks of storage at -20 and -24°C.

Table 1. Detection of *P. phosphoreum* in thawed mince kept at different freezer temperatures and storage periods and further MA-packed prior to chilled storage

Groups	Freezer storage time	@ -19.5 ± 1.2°C		@ -24.3 ± 0.6°C		@ -28.1 ± 2.6°C	
		Detected after *	log/g	Detected after *	log/g	Detected after *	log/g
Control - natural contami-nation (log 1.8/g)	1 day	thawing	0.5	thawing	0.7	thawing	0.7
	4 weeks	7 d	1.0	7 d	2.1	7 d	2.9
	7 weeks	14 d	0.3	14 d	1.5	7 d	0.7
	15 weeks	21 d	na	21 d	na	14 d	na
	52 weeks	**nd**	-	21 d	1.1	21 d	1.6
Lower contam. level (log 2.8/g)	1 day	thawing	0.6	thawing	0.6	thawing	0.9
	4 weeks	7 d	1.6	7 d	2.0	7 d	3.8
	7 weeks	14 d	0.7	14 d	1.5	7 d	1.5
	15 weeks	21 d	na	14 d	na	7 d	na
	52 weeks	**nd**	-	21 d	1.3	21 d	1.1
Higher contam. level (log 6.4/g)	1 day	thawing	4.5	thawing	4.7	thawing	5.0
	4 weeks	thawing	2.8	thawing	2.9	thawing	3.9
	7 weeks	thawing	2.1	thawing	2.6	thawing	3.9
	15 weeks	7 d	2.3	thawing	2.5	thawing	4.4
	52 weeks	**nd**	-	21 d	1.5	14 d	3.6

* Detected after thawing or after further MA storage at chilled temperature (weekly testing)
na: not available due to technical problems with the Malthus conductance apparatus
nd: not detected

Conclusion

Freezing greatly affected *P. phosphoreum*, while *S. putrefaciens* was less sensitive. Temperature of -20°C and prolonged storage (4 weeks and more) had the most drastic effect on the microbial load.

These results are part of a project funded by the Icelandic Research Council (RANNÍS) and the Icelandic companies ÚA and ÍSAGA.

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P 32. COD SPOILAGE FLORA INHIBITION BY CITRIC ACID AND POTASSIUM SORBATE

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Abstract

The dried salted cod market has presented some changes with the introduction of more convenient products such as desalted cod. However, these new products are not as stable as the traditional ones and preservation methods need to be developed to prevent the growth of undesirable microorganisms and to increase its shelf life. One of the most frequently used preservation methods is the addition of food preservatives. Among them, citric acid and potassium sorbate are both generally considered as safe (GRAS) and have been used as antimicrobial agents for decades. The objective of this work was to study the antimicrobial activity of these two preservatives on cod spoilage flora. Their inhibition effect was initially tested on 110 bacterial strains, isolated from desalted cod, and thereafter on refrigerated cod juice and desalted cod inoculated with the dominant spoilage bacteria (*Pseudomonas fluorescens*/*putida* and *Shewanella putrefaciens)*.Microbiological analysis for *Pseudomonas*, hydrogen sulphide producers and total viable counts were performed in days 0, 3, 6, 9, 12 and 15 of cod storage by the spread plate method in Pseudomonas agar and Iron Agar incubated at 30ºC for 48 hours and in Plate count agar with 3% NaCl incubated at 15ºC for 5 days.

Combination of 0.15% citric acid and 0.10% potassium sorbate inhibited more than 60% of the isolates, including the two dominant spoilage bacteria. A total inhibition was observed for *S. putrefaciens* whereas only partial inhibition was noticed for *P. fluorescens*/*putida* both in fish juice and cod treated samples.

Materials and Methods

Preservative solutions were added to tryptone soya broth with yeast extract (TSB-YE), either individually or in combination, originating eight media with different food preservative amounts: TSB-YE1, with 0.07% citric acid; TSB-YE2, with 0.15 % citric acid; TSB-YE3, with 0.05% potassium sorbate; TSB-YE4, with 0.10% potassium sorbate; TSB-YE5, with 0.07% citric acid and 0.05% potassium sorbate; TSB-YE6, with 0.07% citric acid and 0.10% potassium sorbate; TSB-YE7, with 0.15% citric acid and 0.05% potassium sorbate; TSB-YE8, with 0.15% citric acid and 0.10% potassium sorbate. A total of 110 bacterial strains, isolated from desalted cod and identified, by API 20 E and 20 NE systems, Vitek GPI and GNI+ cards, were tested for inhibition by citric acid and potassium sorbate by inoculation in TSB-YE1 to TSB-YE8 broth and incubation at 30ºC for 48 hours.

Fish juice, prepared from cod according to Gram *et al.* (1987), and desalted cod both added with citric acid 0.15% (w/v) and potassium sorbate 0.10% (w/v) were inoculated with two bacteria identified as *Pseudomonas fluorescens/putida* and *Shewanella putrefaciens*, selected among the tested strains due to their spoilage activity, and stored at 4ºC for 15 days. Microbiological analysis for *Pseudomonas*, hydrogen sulphide producers and total viable counts were performed in days 0, 3, 6, 9, 12 and 15 of storage by the spread plate method in Pseudomonas agar and Iron Agar incubated at 30ºC for 48 hours and in Plate count agar with 3% NaCl incubated at 15ºC for 5 days.

Results and Discussion

The amount of growth inhibition of the selected bacterial strains in the presence of each of the eight food preservative solutions/mixtures is presented in Fig. 1. The culture media TSB-YE7 and TSB-YE8, containing respectively 0.15% citric acid + 0.05% potassium sorbate and 0.15% citric acid + 0.10% potassium sorbate, showed a growth inhibition of 39.8% and 61.3%, much higher than the other tested media.

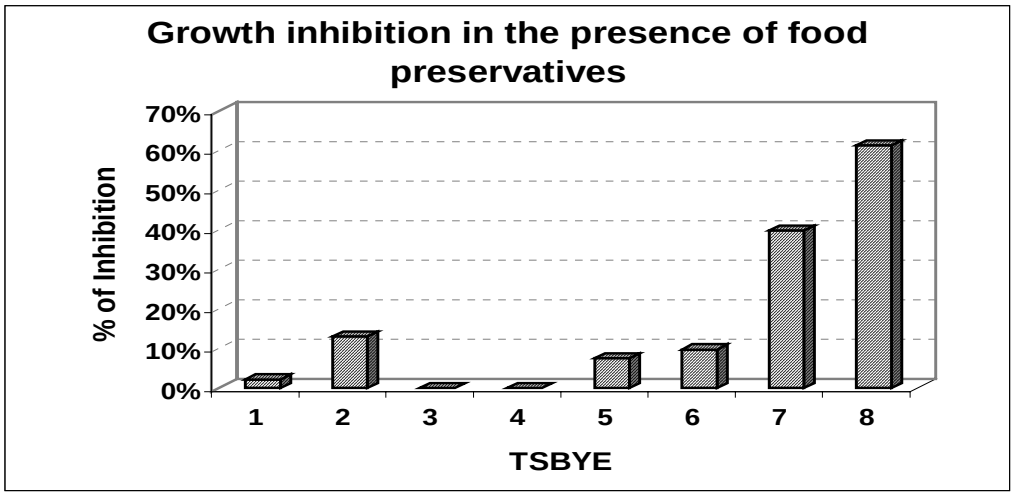

Figure 1 - Microbial growth inhibition in TSB-YE1, TSBY-E2, TSB-YE3, TSB-YE4, TSB-YE5, TSB-YE6, TSB-EY7 and TSB-YE8 broth.

A considerable growth inhibition was observed for both spiked strains, either individually or mixed, in cod juice preserved with citric acid and potassium sorbate. For *Shewanella putrefaciens* a total inhibition was observed in cod juice, whereas for *Pseudomonas fluorescens/putida* a partial inhibition was noticed, with levels near the detection limit.

The growth of *Shewanella putrefaciens* in desalted cod was observed only in preservative free inoculated samples, showing that the tested preservative mixture inhibited the growth of this strain. In the other samples *Shewanella putrefaciens* was not detected, suggesting that this microorganism was not present in the raw material (Fig. 2).

Pseudomonas fluorescens/putida was isolated from all desalted cod samples, either spiked or not (Fig. 3), suggesting that this organism was already present in the raw material. The growth of *Pseudomonas* was slightly inhibited by the addition of the preservatives, indicating the sensibility of *Pseudomonas* to the preservatives used in this trial as already observed by Robach, 1978.

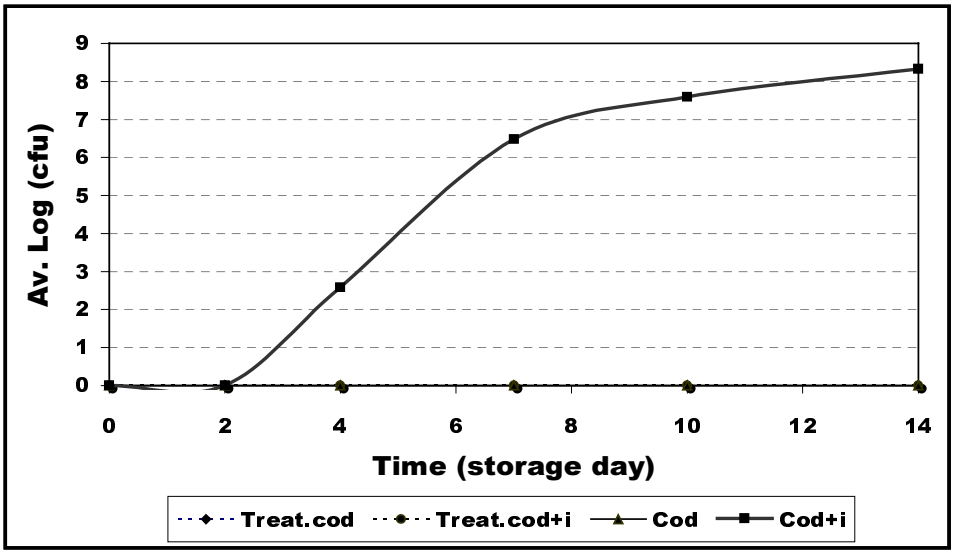

Figure 2 - Growth of *Shewanella putrefaciens* in refrigerated desalted cod: Treat. cod - added with preservatives; Treat. cod + I - added with preservatives and spiked; Cod – control; Cod + I – spiked control.

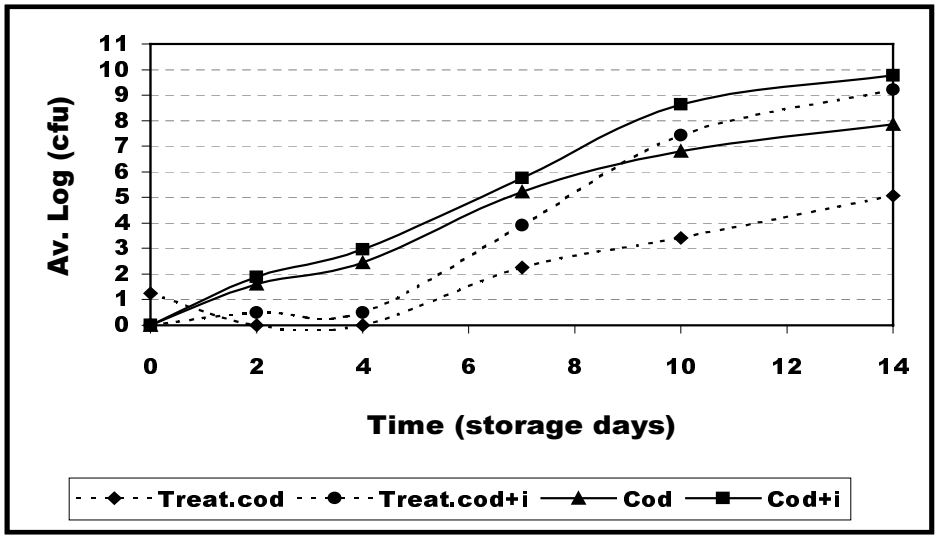

Figure 3 - Growth of *Pseudomonas fluorescens/putida* in refrigerated desalted cod: Treat. cod - added with preservatives; Treat. cod + I - added with preservatives and spiked; Cod – control; Cod + I – spiked control.

Results suggest that application of citric acid and sorbate potassium can contribute for extending the shelf life of refrigerated desalted cod products, as active spoilers can be present in desalted cod produced both from dried and wet salted cod (Pedro *et al*., 2002).

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P 33. *PSYCHROBACTER*: A SPECIFIC SPOILAGE ORGANISM IN CHILLED STORED DESALTED SALT-CURED COD

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Abstract

Salt-cured and dried salt-cured cod (*Gadus morhua*) are important products from the North Atlantic fisheries and primarily consumed in the Latin American and the Mediterranean countries. The fish contains approximately 20% NaCl, and must be desalted (rehydrated) for at least 24 hours before consumption. This process has traditionally been carried out in the households, but changes in consumer behaviour have lead to a demand for ready-to-use desalted products. However, the shelf life of air packed desalted cod stored at 4 °C vary from 2-10 days, depending on the quality of the salt-cured product, hygienic conditions during desalting and desalting method. The desalted cod seems to spoil differently to fresh cod. For instance, characteristic odours related to fresh cod spoilage is usually not noticed. The specific spoilage organisms *Shewanella putrefaciens* and *Photobacterium phosphoreum* are also not present in desalted cod products. Recent studies have shown that *Psychrobacter* is the dominating bacteria in salt-cured and dried salt-cured cod when desalted using high hygienic standards, and this is suggested as a specific spoilage organism in desalted cod.

The aim of this study has been to study the microbiota in various industrially and laboratory produced desalted salt-cured and dried salt-cured products as well as reveal the spoilage potential of *Psychrobacter* in chilled stored desalted products.

After sterile desalting of 38 salt-cured and dried salt-cured cod products the microbiota was found to be dominated (90-100%) by the bacterium *Psychrobacter*. This bacterium is a Gram-negative, oxidase- and catalase-positive, halotolerant, psychrotolerant and facultative aerobe coccobacilli. In the sensory analysis of the chilled stored desalted cod off-odours described as musty and putrid dominated, making the product unacceptable within 7-10 days. Inoculation of samples with high levels ($6.2x10^5$ cells/g muscle) of a reference strain of *Psychrobacter immobilis* enhanced the musty and putrid off-odours, strongly indicating that this bacterium caused the spoilage. *Psychrobacter* showed no proteolytic activity, did not produce H_2S or TMA, but the bacterium was able to hydrolyze lipids. However, no chemically changes were measured in rancidity or total volatile nitrogen during 14 days of storage. The spoilage pattern of desalted cod is therefor clearly different from what is observed in fresh cod. This is caused by limited survival of the normal spoilage biota in fresh fish, due to the high salt concentration in heavy salted products.

In conclusions, the results show that *Psychrobacter* is the main specific spoilage organisms in chilled stored desalted salt-cured and dried salt-cured cod when desalted using high hygienic standards.

Introduction

Salt-cured and dried salt-cured cod (*Gadus morhua*) are important products from the North Atlantic fisheries and primarily consumed in the Latin American and the Mediterranean countries. The fish contains approximately 20% NaCl and hold long shelf life (2 years) at 4 °C. The heavy salted fish must be desalted (rehydrated) for at least 24 hours before consumption. Desalted products are however spoiled within 2-10 days at 4 °C, depending on raw material quality and the hygienic conditions during desalting, probably due to rapid growing bacteria.

Materials and Methods

A total of 38 samples of salt-cured and dried salt-cured cod products, described in Table 1, were analysed. Total bacterial counts were determined on Plate Count Agar supplemented with 3% (w/v) NaCl and total count of *Psychrobacter* by studying colony and phenotypic characteristics as described by Juni and Heym (1986) and Bjørkevoll *et al*. (2003). Spoilage potential was analysed by studying H_2S and TMA production on respectively Iron Agar (Gram et al., 1987) and Growth Medium Broth (Dalgaard et al., 1994). In addition, sensorial characteristics of desalted fish during chilled storage were described by a sensory expert panel of 5 persons at our institute. Development of off-odours were studied by inoculation of $6.2x10^5$ cells/ g muscle of a reference strain of *Psychrobacter immobilis* (CCUG 21770) on desalted samples. The samples were stored chilled and compared with untreated control samples after 0, 7 and 14 days of storage.

Results and Discussion

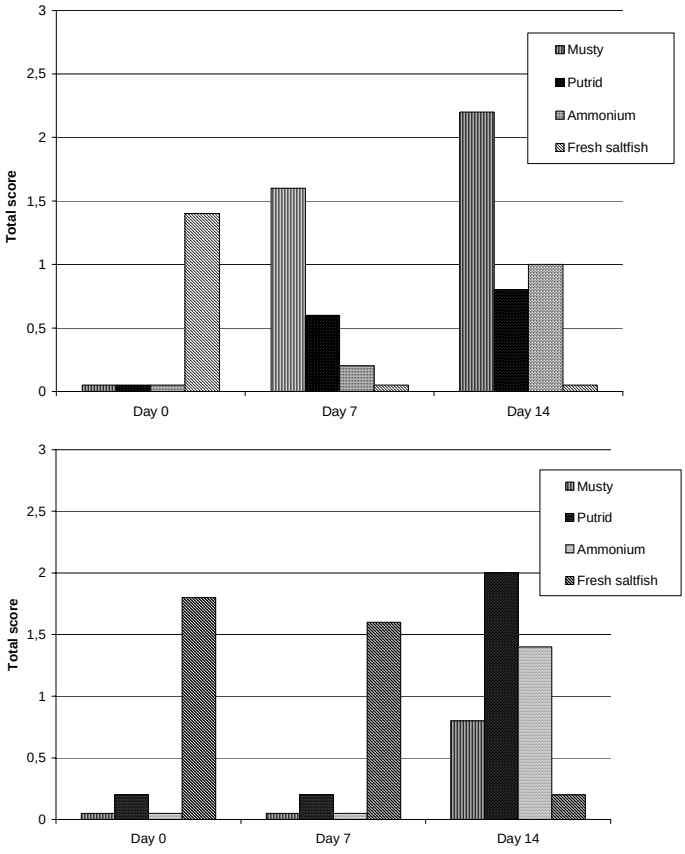
Recent studies have shown that *Psychrobacter* is the dominating bacteria in salt-cured and dried salt-cured cod when the fish is desalted using high hygienic standards (Bjørkevoll *et al*. 2003). After sterile rehydration of 38

samples of salt-cured and dried salt-cured cod products the microbiota in all products was dominated (90- 100%) by the bacterium *Psychrobacter* (Table 1).

Table 1 Salt-cured and dried salt-cured product characteristics and producers name and locality (number of products analysed shown in brackets). Total bacterial counts in rehydrated samples stored for 7 or 5* days at 4 °C and total count of *Psychrobacter* (incubation at 12 °C on PCA with 3% NaCl)

Type of Product	Producer	Bacterial counts (CFU/g)	Psychrobacter (%) of total count
Dried salt-cured cod (4)	Andreas Bjørge A/S, Ålesund	$4.7x10^6$	95
Salt-cured cod (4)	Andreas Bjørge A/S, Ålesund	$4.4x10^6$	95
Salted fillet of cod packed in	West Fish A/S, Ålesund		
- traditional cardboard box (4)		$7.0x10^{7*}$	98
- sealed plastic box (4)		$4.1x10^{7*}$	100
- sealed plastic box with MAP (4)		$4.2x10^{7*}$	96
Salt-cured cod (3)	Tromvik Fiskeindustri A/S, Tromsø	$4.2x10^{5*}$	90
Salt-cured cod (field experiment) (6)	Fiskeriforskning A/S, Tromsø	$6.0x10^6$	100
Dried salt-cured cod (3)	Fiskeriforskning A/S, Tromsø	$4.7x10^6$	100
Dried salt-cured cod (3)	Unknown, Lisbon, Portugal	$2.4x10^6$	95
Salted filet of cod (3)	Unknown, Valencia, Spain	$3.7x10^6$	95

In sensory analysis of the chilled stored desalted cod off-odours described as musty and putrid dominated, making the product unacceptable within 7-10 days. Inoculation of high levels of a reference strain of *Psychrobacter immobilis* enhanced the musty and putrid off-odours, indicating that this bacterium caused the spoilage. Off-odour development is shown in Figures 1 and 2. *Psychrobacter* showed no proteolytic activity, did not produce H_2S or TMA, but the bacterium was able to hydrolyze lipids. However, no chemically changes were measured in rancidity or total volatile nitrogen during 14 days of chilled storage.



Figures 1 and 2 Development in odour characteristics in rehydrated salt-cured cod during 14 days of storage at 4 °C for respectively inoculated (left) and control samples. Score 0 defines absent odour and 3 defines dominant odour.

Desalted cod seem to spoil differently to fresh cod. For instance, odours that are characteristic for fresh fish spoilage are not usually noticed, probably because the specific spoilage organisms in fresh cod *Shewanella putrefaciens* and *Photobacterium phosphoreum* are not present in desalted cod of good quality.

Conclusions

The results show that *Psychrobacter* is the main specific spoilage organism in chilled stored desalted salt-cured and dried salt-cured cod when desalted using high hygienic standards. The spoilage pattern in desalted cod is clearly different from what is observed in fresh cod. The *Psychrobacter* produces off-odours described as musty and putrid that limits the shelf life to 7-10 days.

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P 34. DETERMINATION OF B_6-VITAMERS IN RAW, STORED AND PROCESSED FILLETS OF ATLANTIC SALMON (*SALMO SALAR*)

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Abstract

The term "vitamin B_6" covers several compounds which all have the same qualitative activity as pyridoxine in rats. Phosphorylated pyridoxal and pyridoxamine are known to function as cofactors in several enzymes involved in the amino acid metabolism.

The aim of this work was to study the effects of storage and processing on the loss and interconversion of B_6 vitamers in Atlantic salmon flesh. Batches of salmon fillet from different producers were exposed to processing and storage regimes such as cooking, smoking, vacuum packing, and storage at +4°C and –20°C. Raw fresh fish was included as control. Hydrochloric acid and acid phosphatase was used to extract the vitamers, and the HPLC procedure for separation of the B_6vitamers included an ODS column and fluorimetric detection (Ex.290 nm; Em. 395 nm) with 66 mM KH_2PO_4 (pH 2.1) as mobile phase. This system is able to separate pyridoxine, pyridoxal and pyridoxamine, their respective 5´-phosphates, and pyridoxic acid.

Analysis of fresh salmon fillet from 4 different producers showed a range in total vitamin B_6 concentration between 0,62 and 0,68 mg 100 g^{-1} sample (wet weight), and the relative amount of pyridoxal and pyridoxamine ranged between 57-66% and 32-41%, respectively. Storage resultet in a 4%, 10%, and 15% reduction in total content of vitamin B_6 (vitamin content related to protein content) related to fresh fish for vacuum packed fillets frozen for 8 days, vacuum packed fillets chilled for 2 days, and fillets frozen for 3 weeks, respectively. Smoking of fillets resultet in a 23% loss of vitamin B_6. A shift in pyridoxal to pyridoxamine vitamere forms was measured as a result of chilling, freezing and smoking.

Traceability in the fishery chain - Molecular biology techniques

Keynote 4
Lectures 35-40
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K 4. TRACEABILITY IN THE FISHERY CHAIN TO INCREASE CONSUMER CONFIDENCE IN FISH PRODUCTS - APPLICATION OF MOLECULAR BIOLOGY TECHNIQUES

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Abstract

The increased focus on food safety and the occurrence of food scandals has been the driving force for consumers requesting traceability for foods in general, including seafood. Traceability is defined as the ability to trace the history, application and location of a given product, but in many cases one is only interested in knowing the origin of the product and if it is authentic. In order to decide what should be traceable and what the traceable unit should be, it is necessary to know what the requested information should be used for. Branched chain traceability is the most difficult to deal with. For fresh fish chains pioneering work has been done, showing how traceability could be implemented using information technology and Internet based communication, and the first standards for traceability within chains for captured fish and farmed fish has been developed. The impact of traceability is considered to be essential for improving consumer confidence in seafood and as an aid to provide more sustainable fisheries. Species identification and seafood authentication are considered very suitable areas for introduction of molecular biological techniques into seafood research and technological development. As implementation of traceability will require investments and higher product costs, it is assumed that it will be the larger companies and retailers that will take the lead in the implementation of seafood traceability. The new food regulations to be introduced within the EU in 2005 will include the requirement for food traceability. This will be important for extending its use.

Introduction

The common meaning of the word traceability when applied in the context of food production is taken as the possibility for tracing a feature of a product back in the production chain, and it is being extensively used today. It is becoming a buzzword, and one could believe that the word has been invented for the purpose of today's use. This is not so. According to the extended version of Oxford English Dictionary the word was placed in dictionary terms as early as 1891, and was used for expressing the same meaning as today. An earlier form was 'traceableness', said to be 'the quality of being traceable', a term apparently not being used today.

There is thus not very much uncertainty as to the meaning of the word as such, but why is it being used so extensively today, and how is it interpreted when it is said that 'full traceability' must be possible? When labelling a food product the legislation determines to what extent information about e.g. production date, shelf life, contents, and energy tabulations, etc. shall be visibly printed on the label. The information shall be verifiable, so that control agencies can check the validity of the information given.

The appearance of the product itself, to the extent that it can be inspected the way it is wrapped or packaged, together with the information given on the label, is the information the customer has available in the purchase situation. A lot of the information on the label is not directly verifiable to the customer so it must be trusted that the information is correct. Sometimes the customer would like to know more than is expressed on today's labels. In the typical purchase situation in a supermarket where one is offered a lot of different products, the customer may wonder: Where does this product come from? Is it from my own country, or has it been imported from somewhere else, and in that case, where does it then come from? Information about origin is thus requested, and further may be a request of information on whether the production has been environmentally sound, etc. Product features like this is clearly not visible when looking at the product, or at least only in rare cases, so if such information should be added when labelling, it must be required that it is validated information. It could also be said that the information should be traceable.

The question of where a product comes from may be asked out of mere curiosity, but it is quite clear that a stronger pressure for getting this information occurred after the food scandals where e.g. dioxin was found in certain foods from Belgium in 1999 (Bernard and others 1999), and the BSE crisis started in Britain (Aldhous 2001; Abbott 2001; Pennings and others 2002). These cases are related to food safety and information is requested in order for the customer to be able to avoid certain foods. In other cases, there may also be reasons dictated by e.g. political issues, where the customer does not want to purchase a product from a certain country or region in order to prevent the producer from that particular area to earn money on their products.

In the same way as a consumer wants to know more about the retail product than required by today's legislation, the players in a production chain from the raw material until the retailer may also want to know more about the commodity being handled than is accessible today. This could be a direct result of the request from customers wanting to know more about the product, or it could be information the processor or partner in the chain

wants for his own operation and e.g. for brand protection. Traceability is thus becoming necessary for tracing information and features of a given product at any given stage in the production process.

Each link in the production chain, the final customer and the control agency may have different expectations to traceability. Instead of wanting information about all events during the production, the more limited information of e.g. if the product is authentic or originating from a certain source may be required. Even if the proper information is provided, proof may be needed for control purposes. In such cases, specific methods are needed for checking the authenticity of the sample. Tools are now available within molecular biology for such testing. The development of molecular biological techniques is very powerful, and should be implemented in many areas within seafood research. The opportunity for using methods for checking authenticity is so promising and obvious, that authenticity testing has become an area where molecular biology has penetrated seafood research first. For many people the understanding of traceability within the food area has been limited to authenticity testing only. Authenticity should rather be considered a valuable tool for providing traceability, but the wider comprehension of traceability goes far beyond that.

What should be traceable?

As already said, the origin of a product is becoming information requested more frequently than before. In addition, other information may also be requested. The ISO standard (ISO 2000) defining traceability in the product context contains the following expression about traceability:

"*The ability to trace the history, application or location of that which is under consideration. When considering a product, traceability can relate to:*

- *The origin of material and parts*
- *The processing history*
- *The distribution and location of the product after delivery*"

The three keywords found in the general definition are thus 'history', 'application' and 'location'. When confining it to a product, terms like origin, processing history and distribution after delivery are being introduced.

Each of these terms needs further consideration in order to specify the information requested. When e.g. buying a fish at the fresh fish market, it may be sufficient to know the geographical area from where the fish was captured if provided from traditional fisheries. However, the customer may also want to know when it was caught and by whom. Then further information than a more narrow definition of the word origin is requested. The information on when the fish was caught is usually asked for estimating the quality of the fish, but then the additional information of how it has been handled and stored becomes even more necessary.

The next question will however be to identify which of the parameters during handling and storage are determining the quality of the fish. The temperature by which the fish has been stored will be more important than knowing e.g. the size of the boxes the fish has been stored in. This simple consideration illustrates that it is necessary to know why the information should be retrieved or what it is intended for before one can answer the question of which information should be traced.

When the reason for providing information is known, the process of identifying data to be recorded can start. It is rapidly realised that the amount of data that could be made available may become very large, so it is a question of being able to reduce the amount to only the relevant data. Recording and storage of data just because it is 'nice to know' is not realistic in practical terms, where data recording should be reduced to 'need to know'. On the other hand, relevant data should not be lost. In many situations relevant data are being recorded, but not being stored for traceability purposes. In a given production chain a new consideration is often needed to understand which data should be recorded for which purpose, how the data should be accessed, and for how long it should be stored.

Where and when should data be recorded?

When we know why we want to have certain information and we have identified the necessary data to be recorded, we need to decide where and when we should record the data. The track-and-trace concept should be applied (Børresen and others 2003). This principle says that in order to be able to trace something within a chain, a track must have been laid down first. So the data being recorded and stored represent the track, and in order to make the track continuous, the relevant data must be recorded in all steps and made available, which in most cases mean that it must be transmitted between the different partners in the production chain. Any requested feature can then be traced in the chain to where it originated. Another way of expressing this is to say that one is tracking forwards and tracing backwards. One of the few theoretical considerations and proposal of terms to be used for the exchange of data between each partner has been published by Moe (1998).

Chain traceability as described above is fairly straightforward in a single chain where e.g. a fish is being processed and transported to the market where it is being sold. The situation gets more complicated in branched chains, where the raw material is being split into several parts, and each part moves in different directions. Similarly, a product may be assembled from different ingredients of different origin.

An example could be a white fish caught during the spawning season. The fish is processed by being gutted, the roe taken out, and the fish filleted. The roe may be salted and smoked before being mixed with other ingredients for making a caviar product. The fillet coming from the same fish is going a completely different way, being cut into portions or processed together with other ingredients into a ready to eat meal. The waste fraction

may be processed to animal feed. The fish is thus being split into three different chains, but the basic data must be available for each chain until the point where the split occurs.

Continuing with the same example, the situation becomes more difficult when e.g. the roe product is being produced from roe being obtained from different fishes. It must then be observed if all the roe comes from the same catch or from different catches taken by different boats, maybe in different geographical locations. It must then be decided what should be the traceable unit. Under practical circumstances, reference is usually made to the lot or batch of fish being produced. The same applies for the fillet and for the material being processed to feed.

Achievements within fish chain traceability

The introduction of chain traceability is in its infancy in the fishing industry today. Pioneering work has however been done recently by Frederiksen and Bremner (2001) and Frederiksen and others (2002). In the first study, three Australian and three Danish chains were investigated. The Australian chain concerned fish and crustaceans originating in Australia and transported to Japan, where the products were marketed.

The Danish chains concerned fresh fish caught by vessels outside the Danish coast, and later sold at the Danish domestic market. In the study where both the Australian and Danish chains were considered, interviews were conducted to reveal the possibilities for introducing traceability in the chains studied. A clear interest was identified among the operators in the chains, but only a few were willing to pay for better information than currently received today.

In the second study a Danish fresh fish chain was studied. Equipment for making traceability possible from on-board the fishing vessel until it reached the supermarket was identified and tested. Data was recorded throughout the chain and entered into computers communicating with a central server by a web-based system. When tested it was shown that the chain traceability could be operated from point of catch to the point of sale in the market.

When recording data to be transmitted and used by different partners, it is important that the data can be read and understood by all players. Further, a common understanding of what should be recorded for which purposes should also be decided. If different players would record different data for the same purpose, chaos would soon result and communication would be almost impossible. A concerted action project, 'Tracefish', was supported from the EU Commission with the purpose of agreeing on which data should be recorded and applied in traceability systems for the implementation of chain traceability with electronic means.

Working groups analysed each step in the production chains from traditionally captured fish and aquaculture operations and identified data elements of interest. Each element was described and it was decided into which of three recording categories the element should fall into. The three categories were 'shall', 'should' or 'may' be recorded respectively. The most important data elements for traceability were placed in the 'shall be recorded' category, whereas the other elements were placed in the more optional categories.

The project resulted in the first voluntary standards for traditionally captured fish and for farmed fish respectively. These standards were approved by CEN (European Committee for Standardisation) as CEN Workshop Agreements (CWA) in November 2002. The agreement documents may be amended after receiving comments and suggestions from users. The documents can be accessed at the Internet address www.tracefish.org.

A working group within the Tracefish project also established a set of specifications for information encoding of recorded data and for transmission of such data between units. It was decided that the resulting working paper should not be approved by CEN, but instead be made freely available on the Internet.

Technical solutions for data capture and exchange

In the studies referred to data was collected by recording information on computers. Data concerning e.g. fish species, day and location of capture, time for icing in boxes, etc. was obtained by observation on the site of handling and entered manually into the computer system. Some data may be recorded by measuring different, and relatively simple, parameters like e.g. temperature, weight, time of processing, etc. It may be convenient to record such data in digital form and enter it automatically into the computer system. This will make the recording more efficient and reduce possibilities for human errors when entering data. However, it has to be determined how frequent automatic sampling should be performed.

A lot of equipment already exists for data measurement, but recording systems for traceability purposes should be further developed, like e.g. automatic recording at certain time intervals and feeding the obtained values into computers. The next step concerns the computer systems applied for data storage, transmission and retrieval. Several software systems already exist, and different companies operate as solution providers on the market. Within the traditional fisheries, some of the solutions are coupled to already existing data registration systems for catching fish like the electronic logbook. This information may also be used for electronic auction systems.

The most convenient labelling systems apply the bar code technique for keeping track of the different lots and batches being handled. Different coding and reading systems may be applied, but the EAN-UCC system (European Article Number – Universal Code Council) seems to be the most convenient for global exchange of data and shipping of goods in the most of the world. Further details can be found at www.ean-int.org. One unique feature with this system is the Global Location Number (GLN) being contained in the bar code, identifying each unit being handled.

The information technology applied is developing rapidly, and different electronic 'languages' may be used. A very convenient and quick way of transmitting and retrieving data exists by using Internet based systems. Information may be located on different computers being accessible by different partners, or may be located on a

central computer being accessible by all partners. The last solution is used most frequently, but it may be a matter of negotiation who shall have access to the data, and who has 'ownership' to it.

The bar code labelling system requires a label to be attached to the items being handled, and this may be difficult in the wet and cold environment where fish is being handled. Experience also shows that water on the label may make reading by the laser beam reader difficult and labels may be difficult to attach to surfaces. A better solution would be to apply electronic chips embedded in the material like e.g. fish boxes or containers within which fish is being shipped. Radio Frequency Identification Tags (RFID) already exists, and experiments are done to embed such devices into plastic vats where fish is stored. There are several types, some which can be used for entering data with the necessary information each time it is being used (read/write option), and some with read only possibilities. As it is embedded there is no possibility for attaching information that can be read by humans, so this must be provided by external equipment if required for identification.

The impact of traceability

Once traceability systems have been implemented, transparency is increased in all operations. Some of the information may be sensitive in the business context, and different forms of protection may be needed. This should be negotiated between the partners involved, and clear agreements should be reached.

It should also be thought of how fast information should be made accessible. This could be interesting in cases where correcting actions are needed for improvement of processing efficiency. Further, as action may be required rapidly if e.g. a product defect is detected on the market, and this defect is of such a nature that immediate action is needed. Several incidences have been reported where pieces of glass have been found inside products packaged in glass jars. The immediate action in a retail store where the problem has been reported will be to take the remaining products off the shelf and report back to the producer. It can however not be known immediately if it is an isolated case, or if similar pieces may be occurring in other glasses. The suspicion that this may be the case will be enough for alerting the press, causing severe problems for the company producing the item. If however, the problem can be traced back in the chain to what caused the incident, the damage may be reduced, provided of course that action is taken to avoid the problem from occurring in the future, and communicate this to the public. Traceability may thus be used for brand protection, which may be applicable in a broader term than exemplified here.

Introduction of validated traceability systems will be a valuable tool for providing documented information about features not obviously visible when observing the product as such. Food scandals like the BSE crisis has not been occurring so far for seafood products, but it would be a good idea to be prepared to take action rapidly should anything like it appear. The probability for unwanted contamination would in itself be reduced if traceability systems like the ones described were implemented. It is further known that seafood originating from fish in specific areas may contain more environmental contaminants, like e.g. dioxins, than in other areas. So the application of traceability to improve consumer confidence in seafood is not far from become necessary.

As fish resources available from traditional fisheries cannot be exploited further, it will be necessary to provide a sustainable production. Until now, it appears that the average consumer is not very much aware of whether the fish being purchased is coming from a sustainable fishery or not, but introduction of traceability may result in more focus on the resources. This could lead to a better possibility of protecting species like e.g. the deep-sea fish species from overexploitation (Valdimarsson 2003).

The impact of traceability may thus be an increased consumer confidence in food commodities in general, and for seafood another benefit might be a better management of fish resources worldwide. Concerning farmed fish there may also be better chances for documenting various forms of aquaculture, like e.g. organic farming systems, and it may be possible to document absence of e.g. the use of GMO in the production systems. Within the global trade of fish traceability is connected to quality issues and in addition to implementation of quality assurance systems, traceability is considered necessary to be implemented for import to the major seafood markets in the industrial world (Tall 2001; Goulding 2002).

In addition to using the traceability data for providing information needed as described here, the existing data may represent an excellent source of information for system analysis. Historical data may form time series that can be used for optimising the operations in the production process. They can further be used for developing new production concepts, or the present day production may be modelled and computer simulations may be applied before complete reengineering of the production.

Application of molecular biology techniques for authentication purposes

As was said in the introduction to this paper, different observers may have different expectancies to traceability. In some cases it is more desirable to know the origin and authenticity of a product than the whole process operation. It may also be necessary for control purposes sometimes to check the validity of traceability information. In these cases the use of molecular biology techniques will be excellent.

Authenticity testing of fish products was first introduced by isoelectric focusing of proteins for species identification (Lundstrom 1980). It was however required that the proteins had not been denatured, so other techniques were looked for that could be used for e.g. canned fish. Such methods were found when applying DNA from the fish (Sotelo and others 1993) after being amplified by the PCR techniques (Lockley and Bardsley 2000). The methods are very powerful, but require that gene probes be found that are unique for the specific species being investigated. This can be difficult for e.g. subspecies of tuna fish (Rehbein 1997; Weder and others 2001).

Another limitation is that the methods are only qualitative. They can reveal different species of fish in mixtures, but can only say if a given species is present in an amount above the detection limit or not. The intensity of the reactions observed can be used for semi-quantitative determinations. The most recent development may however solve this problem by applying the so-called Real-time TacMan PCR methods (Mackay and others 2002; Zhan and others 2002). It is needed that a given gene probe is found that can be coupled to a fluorescent dye, such that the intensity is increasing by the number of which the probe is being amplified in the PCR reaction cycle.

The use of another part of the DNA material has been tried by analysing the so-called micro-satellite DNA markers in the cells. This is a material being outside ordinary 'read frames' of the genome. It turns out that small differences in the micro-satellite DNA can be used for discriminating between different populations of fish species. It has thus recently been shown (Nielsen and others 2002) that cod caught in the North Sea can be distinguished from cod caught in the Baltic Sea when analysing the micro-satellite DNA.

Future traceability

From 2005 on the EU Commission is said to implement a new regulation in which traceability will be required for food commodities, and this regulation must be implemented in the whole EU before the end of 2006. The driving force for the introduction of traceability for food from the consumer side seems to be the increased interest for food safety. This is also the case for the authority side, but in addition a better protection against food scandals is wanted. There is a pronounced need for improving consumer confidence in food, and traceability is considered to be a powerful tool in obtaining this.

The other reasons for introducing traceability as described in this paper, where the players in a given seafood production chain may benefit from it, will also be exploited in the future, but this development will probably be slower, as a certain resistance is observed among the players in the seafood production chain operating today's business. Further, implementation of traceability using information technology and computers will require investments, and the operation of the systems may result in increased production costs. These conditions may be acceptable for larger companies or multinational corporations, being very interested in brand protection and the image of the companies among in the public. The larger retailers will also require far better traceability than existing today. The larger companies will thus probably be the ones leading the way for introducing traceability in the food area.

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L 35. TRIAL OF FARMED FISH TRACEABILITY IN JAPAN

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Abstract

This paper presents the fish traceability in Japan and the economical traceability systems for farmed fish that is under development by authors. In Japan, food industry has lost consumer's trust by doing improper labeling the manufacturing date, the landing port and adaptive material.

The Ministry of Agriculture, Forestry and Fisheries of Japan has been examining the fishery policy concerning the safety of food. The safety of medicine, ingredients, farm environment, imported marine products etc. is now being discussed by the fishery industry, consumer as well as the governmental agencies.

The traceability is discussed as part of the marine products safety securing. Some fishery organizations producing the farmed oyster and surimi are studying the chain traceability for their products. A fishery organization tries to be enacted the farmed fish recording standard scheme, and have a plan to diffuse the use of standard for fish farms. Many traders and mass retailers request more information than the standard scheme and conform to their requirement for suppliers.

Authors have been examining the adoption of additional remarks matched to recognize individual fish over and above invisible 'information' and are planning to develop an economical farmed fish traceability systems which contain the advantage of TRACEFISH program studied by EU and enable the traceability investment for many medium and small sized fishery enterprises.

Introduction

The Ministry of Agriculture, Forestry and Fisheries made the traceability introduction guideline in spring, 2003. The traceability is obligated by a part of food product such as beef. It is arbitrary whether that enterprise introduces the traceability of other foods. The form of the traceability system is also arbitrary. Therefore, the paper-based system is appeared same as a system such as digital code.

The movement of the de facto standard plan is still under proceeding because there are neither a group nor an enterprise that takes the initiative at all, modeling the compound circulation is difficult each fish species and various commercial practices are varied in accordance to each region. Though any industry in Japan is the same. Many people commit that he agree with the plan in general, but would leave miner on details for further discussion.

Food Policy Section, Department of Comprehensive Food, Ministry of Agriculture, Forestry, and Fisheries advertised for safety and quality issues in food industry to develop the traceability system of Japanese version in 2002. The demonstration of the traceability at seven agricultural products and marine products was done. A similar demonstration is planned even in 2003.

The two out of seven was demonstration of marine products.

The traceability demonstration of the farmed oyster was conducted in the winter of 2003. The Food Marketing Research and Information Center Japan acted as the leader.

Miyagi Pref. own about 30% of farmed oyster in Japan. The Shizugawa fishery cooperative transports the oyster to the processor in Matsushima. The oyster arrives in the evening, stuffs packing every 100 gram-400 grams within an hour or two hours. The label that prints the packing number of 11 digits per about 2000 packings a day was pasted for this demonstration. The numbers of 11 digits are information such as the farms, the processing sites that open the shell the producer and the husk and the processing days. To prevent the misrepresentation by doing the product mixture from another aquaculture area, retailer were able to evaluate a comparison between the gross weight of arrival of oyster and the amount of the shipment.

Farmed fish traceability implementation planning

We have a farmed fish traceability implementation plan. When the system could happily developed, it is possible to utilize with information supply chain from farmers to the processors, the wholesalers, and the retailers.

We are proposing for globefish farmers in Nagasaki Pref. traceability system. The amount of the globefish production farmed in Nagasaki Pref. is the top of Japan. However, they are aware of incoming hard competitor with cheep price imported globefish from over seas, that is why they are interesting in development of traceability system so that they can accomplish the bland image. We aim at constructing the system that we cans show you a partial demonstration in a year. Afterwards, we have a plan to introduce a full-specified traceability system.

The landed quantity of the horse mackerel and the mackerel in Nagasaki Pref. is the top of Japan. A large amount of horse mackerel and the mackerel go to the consumer market in fresh raw fish or the processing through

various routes. After completion the traceability system with the globefish, we shall expand with own effect to the mackerel and the horse mackerel.

Unfortunately, the label misrepresentation of food happens frequently. Therefore, majority of consumers would not trust the label in described on food. The following display technology is proved to regain the trust on display.

We will create the technology for identification of a fish. The bar code is ultimately given to the body of fish.

The temperature will be recorded through the supply chain. The checkable technique of attached information is developed with recorded temperature. The temperature was collected in the fishing port and in the transportation from the producing district to the consuming region. We have got information about the date and time of dispatch and the date and time of reception from the sudden change of temperature in memory. Useful for the credibility of information is confirmed.

We will take a picture of fish by the mobile phone with CCD widespread in Japan, and measure a characteristic of fish's body. The expert can be easily identified of the landing season and region from image.

Authors, thus, do utmost to develop the system, introducing, operating as low cost as possible, so that processors, farmers in medium and small size companies are entitled to get benefit to use the system for upgrading the BRAND. Therefore we will use the following standards or ready-made technology shown Table 1.

Table 1 The standards or ready-made technology

	The standards or ready-made technology
Data element	The farmed fish recording standard scheme, TRACEFISH, The UCC/EAN-128 code The standard code for EDI (fish name, producing district, how to tighten, processing method, and etc.)
Data transfer	TRACEFISH, the UCC/EAN-128 code, EDI, XML, XML-EDI, web-EDI , mobile phone with CCD ,IC tag, temperature recording device

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L 36. DISCRIMINATION AMONG SPECIES AND STOCKS OF REDFISH, COD AND HERRING BY CHEMOMETRY OF THE FATTY ACID PROFILE IN SELECTED TISSUES.

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Abstract

The composition of fatty acids in selected tissues of three redfish species, two populations of cod and two populations of herring was determined by a chemometric method, consisting of methanolysis, gas chromatography of the resulting fatty acid methyl esters and multivariate, statistical treatment of the analytical data. The two reared stocks of cod from the Faroe Bank and the Faroe Plateau had significantly different fatty acid profiles in total lipids and in phospolipids from heart tissue. This difference is expected to be genetic and free of biotic and abiotic impacts on the fatty acid profiles due to the fact that the cod were reared for 3 years and 8 months under identical conditions before analysis (Joensen et al. 2000). The three species of redfish from waters off the Faroe Islands and Norway had significantly different fatty acid profiles in heart-tissue, gill- tissue and skull-oil. The fatty acid profiles appear to be species specific. The mutual relationship between *Sebastes marinus* and *S. mentella* is closer than the relationship between either of them and *S. viviparous* (Joensen and Grahl-Nielsen 2000, 2001). The fatty acid profile in the heart tissue of *S. mentella* from eleven locations in the waters off Norway, Faroe Islands, Iceland and from two strata at different depths in the Irminger Sea (Figure 1) indicate the presence of four separate stocks of this redfish species in the North East Atlantic Ocean (Figure 2). The difference between the four groups in pairs was statistically significant ($p < 0.01$). Herring from the North Sea stock and the stock of Norwegian Spring Spawners were distinguished by way of the fatty acid profile in the filet (Figure 3). A new method for stock identification on the individual level is established.

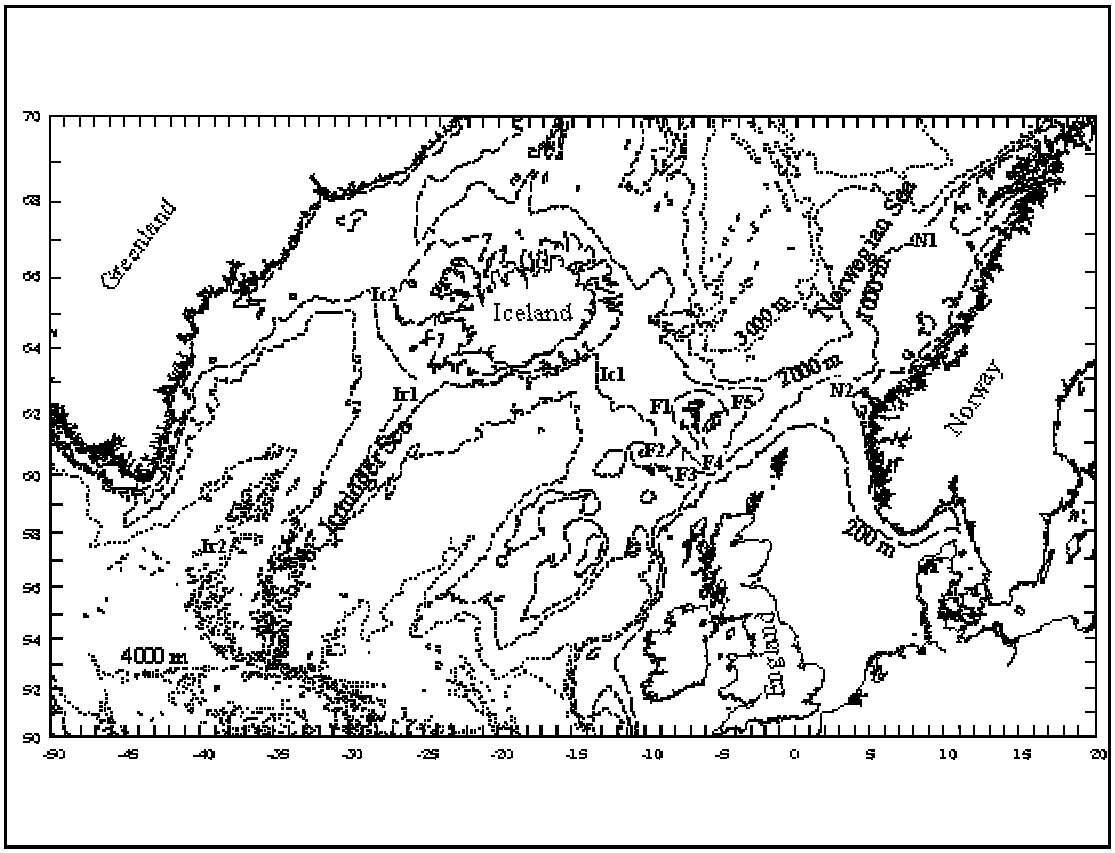

Fig. 1. North-East Atlantic Ocean and the eleven positions where sampling of *S. mentella* took place.

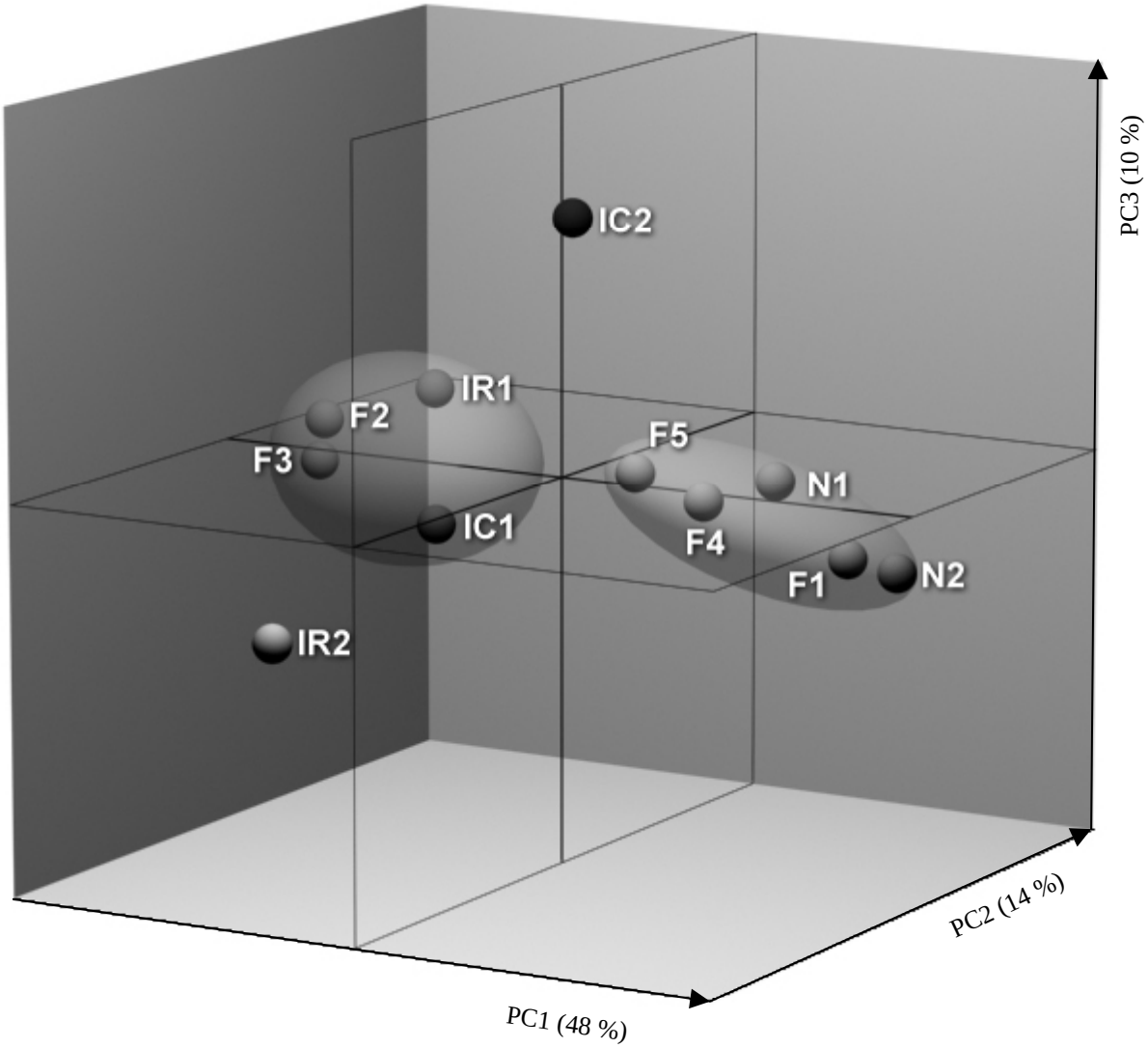

Fig. 2. Three-dimensional PC-plot of *S. mentella* based on the composition all of the 49 variables (fatty acids mostly) in the heart tissue. Each symbol represents an average value of 20 samples from each of the 11 locations in the North Atlantic. The percentage of the total variance along each of the principal components (PC) is given.

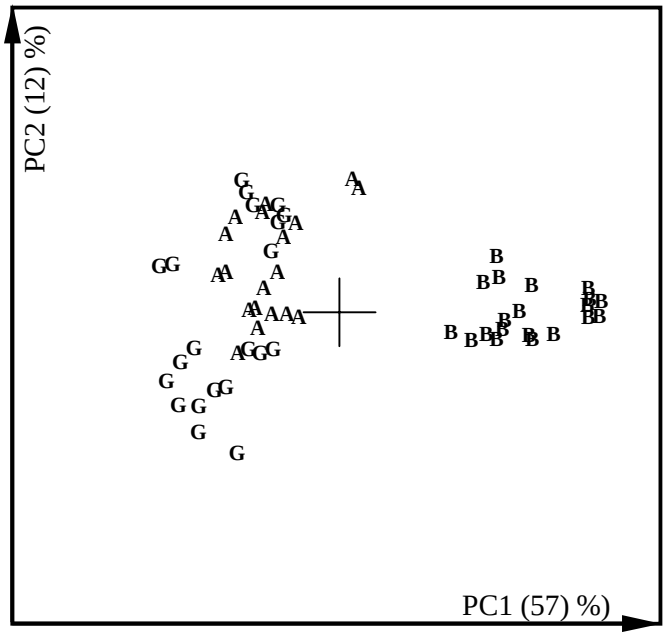

Fig. 3. *Clupea harengus*. Two-dimensional PC-plot of two stocks of herring: Norwegian Spring Spawners (NVG) and North Sea Stock (NS) based on the fatty acid composition in filet-tissue. **A** and **B** symbolize the test herrings, while **G** symbolize the reference NVG-herring. Two parallels from each of 3 x 10 herring are shown. The percentage of the total variance along each of the principal components is given.

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L 37. DETERMINATION OF THE COMPOSITION OF MULTI-SPECIES FISHERY PRODUCTS BY PCR-BASED TECHNIQUES

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Abstract

A considerable number of fishery products contains muscle or other tissue of more than one fish species. Examples are fish cakes, pies, pastries, soups, baby food products, and also fish meal. Identification and quantification of the different species of a product is a very difficult task, as the biological characteristics are lost during processing, and proteins or metabolites may be denatured or degraded.

DNA-analysis based on the polymerase chain reaction (PCR) offers the possibility to identify fish species also in heavily heat treated (e.g. sterilised) products by amplifying and characterising short fragments of DNA. For the development of quantitative PCR methods we have performed the following analyses: (i) Determination of the total DNA content of fish and fishery products, (ii) PCR of nuclear genes for salmonid, gadoid and scombroid fish species, (iii) establishing PCRs being specific for *Pollachius* spp.and *Thunnus* spp., (iv) determination by using real-time PCR of the copy number of a parvalbumin gene in muscle of Atlantic salmon (*Salmo salar*), of the calmodulin gene in yellow fin tuna (*Thunnus albacares*), and of the calmodulin and myostatin genes in muscle of saithe (*Pollachius virens*).

The latter method can be applied to detect and quantify saithe in products like fish cakes, salmon substitute and fish meal.

Introduction

A considerable number of fishery products does contain muscle or other tissue of more than one fish species. Examples are fish cakes, pies, pastries, soups, baby food products, and also fish meal. Identification and quantification of the different species in a food or feed product is a very difficult task, as the biological characteristics are lost during processing, and proteins or metabolites may be denatured or degraded.

PCR-based DNA-analysis offers the possibility to identify fish species also in heavily heat treated (e.g. sterilised) products by amplifying and characterising short fragments, 100-200 base pairs in length, of DNA (1). Quantification of targets (sequences of RNA or DNA) has become much easier and faster with the real-time PCR compared to competitive PCR (2).

Here we report on the determination of gene copy numbers in fish and fishery products by using the Light Cycler Fast Start DNA Master SYBR Green I method, and how to calculate the amount of fish in a product by using these data.

Materials

Authentic fish samples were collected at cruises of the fishery research vessel Walther Herwig III in North Atlantic waters or obtained from other research institutes. Several samples of baby food, hot-smoked eel and caviar were purchased from retail shops in Hamburg.

Methods

For the development of quantitative PCR methods we have performed the following analyses:

- Determination of the total DNA content of fish and fishery products by a fluorescence enhancement assay using the dye Hoechst 33258 (3)
- PCR of nuclear genes for salmonid, gadoid and scombroid fish species.
- Establishing PCRs being specific for *Pollachius* spp.and *Thunnus* spp.
- Determination of the copy number of a parvalbumin gene in muscle of Atlantic salmon (*Salmo salar*), of the calmodulin gene in yellow fin tuna (*Thunnus albacares*), and of the calmodulin and myostatin gene in muscle of saithe (*Pollachius virens*), by real-time PCR using the Light Cycler Fast Start DNA Master SYBR Green I method (Roche).

Results and Discussion

Determination of the total DNA content in fish muscle and eggs

The dye Hoechst 33258 reacts specifically with AT-rich regions of DNA forming a fluorescent complex. RNA and protein do not bind the dye under the conditions of the assay.

The DNA content of fish fillet, eggs and whole fish is given in Table 1. The DNA content of a single grain of sturgeon or trout caviar was determined to be about 6 µg.

Table 1: DNA content of fish fillet, eggs and whole fish

Fish species	**Type of tissue**	**DNA content (µg/g wet weight)**
Haddock, *M. aeglefinus*	Whole fish	147
Whiting, *M. merlangus*	Whole fish	155
Whiting	White muscle	63
Whiting	Red muscle	304
Saithe, *Pollachius virens*	**Whole fish**	268
Cod, *Gadus morhua*	Whole fish	257
Herring, *Clupea harengus*	Whole fish	295
Sprat, *Sprattus sprattus*	Whole fish	281
Atlantic salmon, *Salmo salar*	Fillet	261
Sevruga, *Acipenser stellatus*	Caviar (egg)	450
Osietra, *A. gueldenstaedti*	Caviar (egg)	244
Beluga, *Huso huso*	Caviar (egg)	236
Rainbow trout, *O. mykiss*	Caviar (egg)	85
Eel, *A. anguilla*	Fillet of hot smoked eel	180

Evaluation of PCR assays for nuclear genes

Calculation of the mass of fish fillet from gene copy numbers determined by PCR is only possible on the understanding that the number of gene copies is invariable for different parts of the fillet, and not influenced by biological conditions. As this assumptions is fulfilled better by single-copy nuclear genes than by mitochondrial genes (4), published PCR systems for nuclear genes of fish were tested for their suitability for salmonid, gadoid and scombroid fish species. Finally sequences from the parvalbumin and calmodulin genes were chosen, which could be amplified in a number of species of interest. These amplicons were used to construct species specific, as well as consensus primers, by cloning and sequencing.

Determination of gene copy numbers by real-time PCR

Purified amplicons were used as standard for determination of gene copy numbers in fish muscle. The results obtained for different genes are summarised in Table 2. The numbers are in good agreement with figures calculated from the total DNA content of muscle divided by the DNA content of the haploid chromosome set (5). Copy numbers, as determined by quantitative PCR may be used for calculation of the amount of raw fish within a sample, if the yield of DNA extraction has been taken into consideration. In processed fish, however, the gene copy numbers may be lower due to degradation of DNA.

Table 2: Number of gene copies in raw fish muscle (ww = wet weight)

Fish species	**Gene**	**Copy number/g ww (by PCR)**	**Copy number/g ww (by DNA content)**
Atlantic salmon	Parvalbumin	1.0×10^8	1×10^8
Saithe	Calmodulin	1.1×10^8	$0.5\text{-}1 \times 10^8$
Saithe	Myostatin	0.6×10^8	$0.5\text{-}1 \times 10^8$
Yellowfin tuna	Calmodulin	0.8×10^8	No data
Cod	Calmodulin	0.5×10^8	$0.5\text{-}1 \times 10^8$

Determination of the portion of a fish species in mixed products

In case of products consisting of several fish species, the share of each species may be of interest. This problem can be addressed by measuring copy numbers of a DNA sequence specific for each species and the copy number of a consensus sequence. From the ratio of these numbers the portion of each species can be calculated and the problems caused by DNA degradation and varying yields of extraction are reduced to some extent. However, if

the extractability of DNA is different for the components of a mixture, this method gives erroneous results. As an example of this approach results obtained for determination of the saithe content in baby food are shown (Table 3). Saithe-specific primers amplified a 101 bp sequence of the calmodulin gene, and general fish primers were used to amplify a 90 bp sequence (consensus system) of the myostatin gene (6).

Table 3: Determination of saithe content in baby food. Numbers of gene copies refer to ~ 6 ng of DNA. On the average 1.5 µg of DNA was extracted from 1 g of baby food.

Ingredients, as given on the label	**Gene copies for calmodulin**	**Gene copies for myostatin**
1) Tomatoes, rice, saithe (8.8 %)	145 (13 %)*	1155
2) Noodles, tomatoes, saithe (9 %)	20 (5 %)	428
3) Tagliatelle, brokkoli, saithe (9 %)	183 (53 %)	348
4) Vegetables, potatoes, saithe (8 %)	39 (4 %)	1006
5) Vegetables, potatoes, saithe (10 %)	26 (6 %)	403

* Ratio of saithe to fish

-4-

Real-time PCR gave the following results: (i) In product no.1 and 3, the portion of saithe was much lower than indicated. (ii) In case of these two products beside saithe also other species must have been processed. (iii) Products no. 2, 4 and 5 were mislabelled, because significant amounts of saithe, which should be the only species in these products, could not be detected.

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L 38. AUTHENTICATION AND CONFIRMATION OF PRODUCT CLAIMS

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Abstract

The label of foodstuffs has to be informative, true and – obviously - not be misleading. Fishery products are usually considered healthy foodstuffs with beneficial effects which include high protein digestibility, high content in minerals (iodine, selenium), vitamins, amino acids and derivatives, and polyunstaturated fatty acids (PUFAs). This makes them a choice component in diets designed to be healthy and prevent the development of cardiovascular diseases and cancer. However, the true beneficial effects of marine foods are dependant on the raw material itself and on the degree of freshness and processing: the same material may be highly nutritious (high quality proteins, polyunsaturated fatty acids and rich in taurine, betaine salts and other beneficial osmolytes) or straightforward hazardous (free radicals from lipids and proteins, lack of osmolytes and diet-induced depletion of our own pool, etc.), depending on the processing conditions to which it may have been submitted. Therefore, in order to assess the correct labelling of a product, and specially its health-related claims, we need methods to attest not only the identity and origin of its components but also the processes to which it has been submitted. Our presentation will show some of our results dealing with analyses relevant for the correct labelling of foods, nutraceuticals, and for authenticity issues.

Freshness of the raw material may be important to estimate its allergenic potential and the amount and quality of the proteins. Dory *et al.* [1998] showed that the number of IgE-reactive bands and intensity of the reaction in cod extracts was greater if the fish had been stored for several days than if the extracts had been obtained from post-rigor fish immediately after *rigor mortis* resolution. We used two-dimensional electrophoresis to assess the effect of processing on the protein profile and found a spot of identical apparent molecular mass and pI to that of Gad cI in extracts made from pre-rigor cod. The spot was much smaller in extracts from post-rigor cod. Two dimensional electrophoresis was also used to illustrate how much faster myofibrillar proteins degrade in Arctic compared to tropical species and to identify markers that permit to estimate very early protein degradation [Martinez *et al*., 2001]. Low protein quality, for example due to heat processing, is considered to decrease the absorption of taurine from the gastrointestinal tract. Protein extractability can be used to estimate the heating temperature in some species [Dr Rehbein, personal communication] but not in others [our unpublished results].

Osmolytes, such as taurine, are important to preserve not only the taste of foods but also the biological function of proteins and membranes. Which osmolytes are present and their relative amount are characteristic for each organism, and some species have very high content in taurine, for example *Calanus*. Interestingly, freezing and thawing *Calanus* induced major losses of free amino acids and a complete depletetion of taurine [Overrein *et al.*, 1999]. Our major source of taurine is the diet. Taurine is important because it regulates an unusual number of biological phenomena, including heart rhythm, contractile function, blood pressure, platelet aggregation, neuronal excitability, body temperature, learning, motor behaviour, food consumption, eye sight, sperm motility, cell proliferation and viability, energy metabolism and bile acid synthesis. It has been used as a therapeutic agent in cardiovascular diseases for over 40 years and it is now being used in infant formula, nutritional supplements and in energy promoting drinks. Interestingly, nuclear magnetic resonance analyses have shown the presence of taurine and phosphoethanolamine in high levels in extracts of breast cancerous tissue in humans [Gribbestad *et al.*, 1994, 1999] and in chemically induced tumors in rats analyzed by high resolution magic angle spin (HR-MAS) NMR [Optun, personal communication]. It is possible that this rise in taurine may be an endogenous defense mechanism against tumor proliferation. Methods such as NMR, suitable to demonstrate the content of osmolytes in fish products must be of relevance to their labelling as healthy products.

PUFAs (DHA and EPA) are essential components for any diet claiming to be healthy. As taurine, they protect against cardiovascular diseases and inhibit growth of some cancer cells [Heimli, Svanhild]. We are currently examining whether the lipid profile of chemically induced tumors keep a relationship with the lipid profile of the diet [Optun, *et al.*, on going research]. The profile of fatty acids of edible tissues in fish is practically a reflection of the components of its diet: not only can the fatty acid fingerprint be used to discriminate wild from cultivated fish but also to identify the lipids included in its diet (from animal or vegetal origin, soya oil, etc). Similarly, nuclear magnetic resonance used to obtain lipid fingerprints of some nutraceutical fish oil capsules showed them to be incorrectly labelled regading the species and the lipid content.

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L 39. DEVELOPMENT OF AN IDENTIFICATION AND QUANTITATION SYSTEM FOR COD (*Gadus morhua*) USING TAQMAN ASSAY

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Abstract

This work has been carried out within the framework of the EU project QLK1-2000-00476 (development of molecular genetic methods for the identification and quantification of fish and seafood, DNAIQ). One of the main objectives of this project is the development of fast and simple methods of identification and quantitation of fish species in seafood products. One of the target group of species are gadoids, and among gadoids, the cod (G*adus morhua*) has a high commercial value and can be present as ingredient in several seafood products.

Real-time PCR allows the quantification of target DNA by using fluorescent labeled probes (Taqman probes) hybridizing to an internal region of a PCR template DNA. Hybridization of the labeled probes and their digestion, as Taq polymerase synthesizes DNA, provokes fluorescence emission from the fluorescent dye attached to the probe. This fluorescence signal can be correlated with the initial DNA target. Taqman assays were employed for developing a specific and quantitative system for *Gadus morhua*. Main problems related with quantitation of cod, such as DNA extraction and standard curve reproducibility will be discussed. The design of a Taqman probe for G*adus morhua*, reaction optimization, specificity and quantitation results, both in DNA and muscle fish mixtures, will be presented.

Introduction

The use of biochemical markers, such as proteins and nucleic acids, has provided a tool for controlling the compliance with labeling regulations of fish products belonging to different families (Bossier, 1999; Mackie et al., 1999). Identification of seafood products using DNA analysis techniques has been widely demonstrated (Sotelo and Pérez-Martín, 2002). However, although identification is a very important issue for labeling, another important issue is to reveal the presence of mixed components in a product and most methodologies will not allow this or the quantitation of particular species in mixed products.

Real time PCR is based in the use of a specific probe, labeled with a fluorochrome, and which attach to one of the DNA strands specifically during a PCR cycle. The attached probe does not emit fluorescence, however when hydrolyzed by a special DNA polymerase (with 5' nuclease activity) the fluorochrome is freed and emits upon excitation with a laser light. The fluorescence is registered by a CCD camera and this signal is correlated with the initial amount of template. The methodology has been used for the determination of quantities of genetically modified organisms (GMO) in food (Wurz et al., 1999).

Gadoids is the common name used for a group of bony fish, included in the taxonomical order Gadiformes, comprising several families with great commercial interest. The family Gadidae comprises mostly commercial species, including Atlantic cod (*Gadus morhua*), pollack (*Pollachius pollachius*) and haddock (*Melanogrammus aeglefinus*). The over-exploitation of cod has led to the collapse of some stocks, especially in the Western Atlantic, and this has prompted the issuing of a moratorium in this fishery. Obviously, the consumer interest for some of these species has not decreased and therefore the market price has increased according to demand. Gadoid species can be found in markets in different presentations, depending on the country. Presentation may include fresh or frozen fillets, smoked, salted, surimi-based products, fish cakes etc. European Union labeling regulations (EC No 104/2000) specifies that the commercial and scientific names should be included on the label of seafood products, however most of these processes involve the removal of morphological characteristics hindering the process of species identification.

The objective of this work is the development of a suitable methodology to identify and quantitate cod (*Gadus morhua*) using real time PCR methodologies.

Materials and Methods

Authentic gadoid species and commercial fish samples

Whole specimens of authentic species were obtained fresh in the local fish market, other specimens were obtained frozen from fish and food industry.

DNA Extraction

DNA extraction from frozen or salted muscle, previously thawed, was carried out using the standard Wizard™ DNA Clean-up System (Promega, Madison, WI, USA).

PCR amplification and sequencing of DNA from Gadoids

The primers used amplify a region of 464 bp of the cytochrome b previously described by Kocher et al. (1989). The sequence of the primers is: H15149AD: 5´-GCICCTCARAATGAYATTTGTCCTCA-3´ for the forward primer, and for the reverse L14735: 5´-AAAAACCACCGTTGTTATTCAACTA -3´ as described in Calo-Mata et al (2003). Sequencing of both strands were performed as described earlier (Calo-Mata et al., 2003).

Taqman probe design and Real time PCR

Multiple sequencing alignments of H15149-L14735 fragment (413 bp) from gadoids were used for selecting the cytochrome b region for probe design. The sequence was loaded in the Primer Express™ 2.0 software and primers and MGB probe were designed in the selected location, following the guidelines given by the software.

DNA extracts from three different fresh cod individuals were prepared after homogenizing the white muscle with a pre-cooled WARING BLENDOR for 1 min. DNA extraction was performed as specified above. Quantitation of DNA in extracts was performed by UV absorption at 260 nm. Primers and MGB Taqman probes were from Applied Biosystems. Amplification was carried out in MicroAmp Optical 96-well reaction plate from Applied Biosystems in a 5700 GeneAmp sequence detection system. The thermal profile employed during the experiments was the following: 95º 10 min, and 40 cycles with 95ºC 15 s and 60ºC 1 min.

Results and Discussion

*DNA extraction from cod***.** One of the requirements for quantitation is the existence of constant ratio between target DNA and tissue.

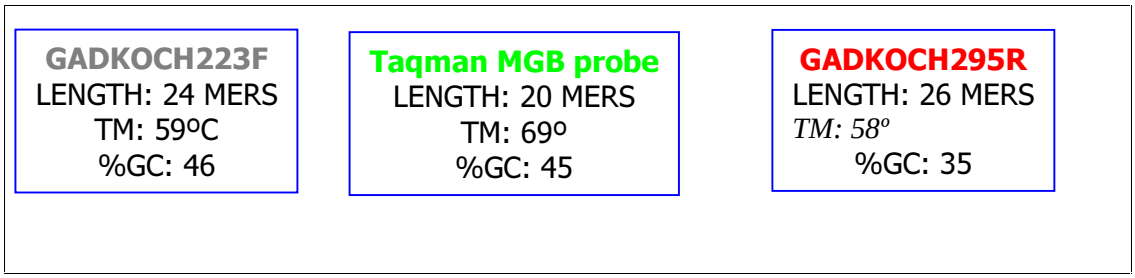
Evaluation of the reproducibility of DNA extraction is one of the main tasks to determine the feasibility of a quantitation system for fish. Three cod specimens, of similar biological characteristics (similar size and weight), were obtained. Fresh white muscle from each specimen was homogenized using a Waring Blendor with a cooling jacket device. DNA was extracted from three replicates obtained from each individual, the average DNA obtained was of 98.14 µg/g of wet tissue with a standard deviation of 28.4.

Probe design. Sequences from different gadoid species were obtained and aligned to search for Cod diagnostic positions within the fragment. The aim was to select a region to design PCR primers which flank a stretch of DNA containing a specific position for cod permitting the design of a Taqman MGB probe.

A region located around 260 bp away from the 5' end of this sequence showed a conserved C which was not present in most of the other gadoids. This region was selected for designing a Taqman MGB probe and the corresponding set of primers.

Polymorphic position was placed in the middle of the probe, and the program (Primer Express V2.0) was forced to find the matching primers. All the conditions recommended for the designing of primers and probes were met: long runs of nucleotides avoided, Tm of primers and probe were between 58 and 60ºC, and the GC content between 30 to 80%. Primers were placed as close to the probe as possible without overlapping it.

Features of the designed primers and probe are shown below.



The amplification with primers and probe with different cod DNA loadings gave a typical amplification profile (Figure 1) with highest Ct values with increasing concentration of template DNA.

The inclusivity test showed that all specimens of gadoid tested (5 individuals) gave a strong signal, whereas the signal given by other gadoid or fish species is negligible.

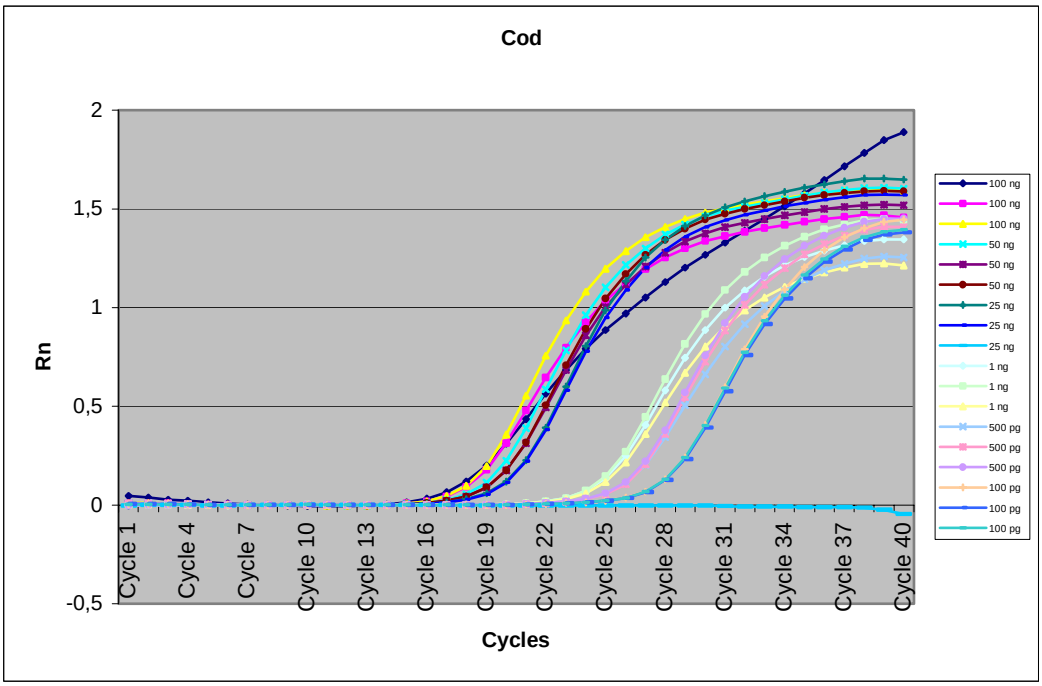


Figure 1. Linear amplification plot of different cod DNA load (100 ng-100 pg)

*Quantitation of cod with Taqman probe***.** Serial dilutions of DNA, ranging from 100 ng down to 100 pg of DNA were prepared. Reproducibility was highest at concentration values 100 ng, 50 ng and 25 ng, whereas at 100 pg the Ct reproducibility was the lowest (Figure 2).

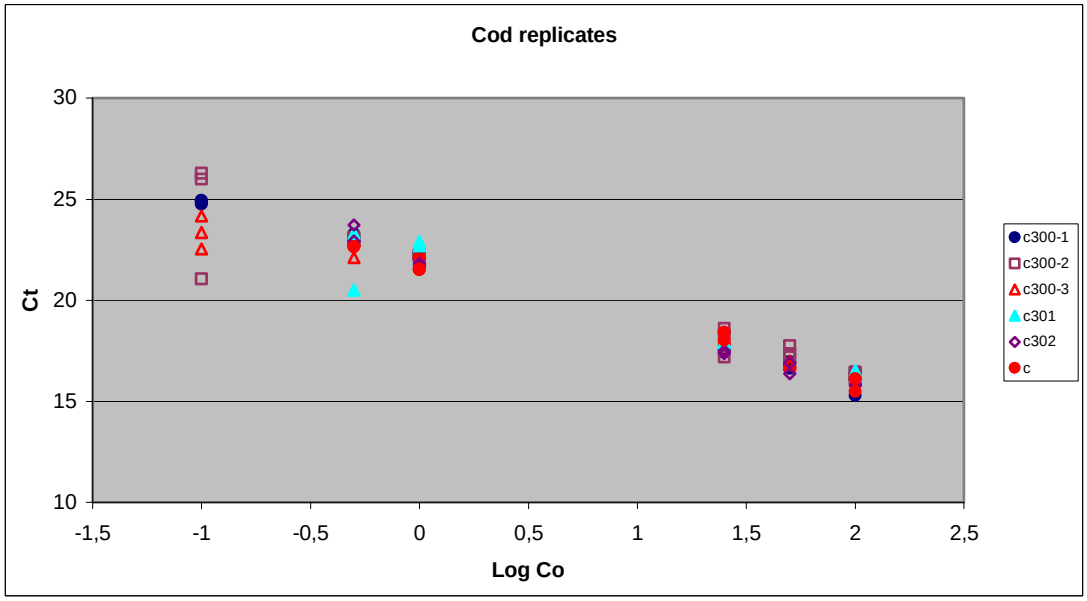


Figure 2. Reproducibility of standard curves prepared from different DNA extracts and specimens of cod. Log Co: log of DNA concentration.

Quantitation of cod in a binary mixture of cod (Gadus morhua) and hake (Merluccius merluccius) DNA. The total amount of mixed DNA used in the Taqman assay was 50 ng. The results showed that Ct values were linearly correlated with percentage of cod DNA in the mixture (Figure 3), indicating that only the cod DNA was detected by the system.

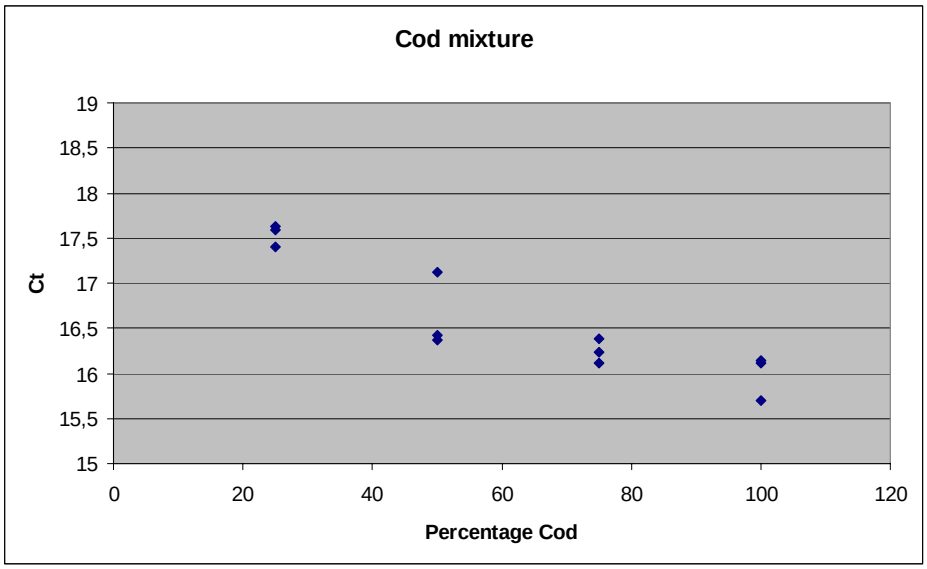


Figure 3. Graphic display of Ct versus percentage of cod in the DNA binary mixture.

A DNA standard curve was prepared using DNA extracts from three cod specimens (data not shown). Using this standard curve, the amount of cod DNA present in the binary mixture was calculated, the real and determined amount of cod DNA are graphically presented in Figure 4. The error obtained in the determination ranged from as low as 0 up to 50%.

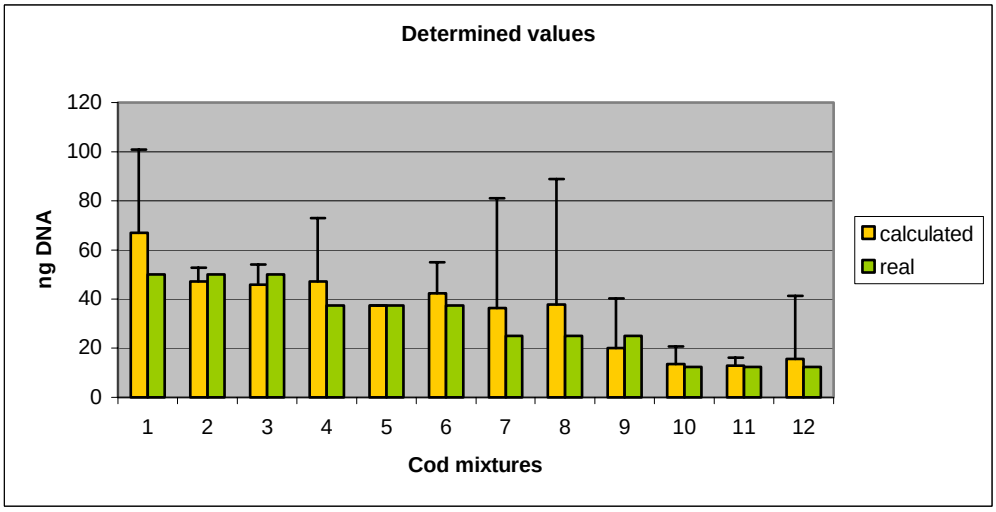


Figure 4. Graphic display of determined and real ng of cod DNA present in the binary mixture cod and hake. 1, 2, 3: 100%; 4, 5, 6: 75%; 7, 8, 9: 50%; 10, 11, 12: 25% of cod DNA.

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L 40. EVALUATION OF IMPACT OF SMOKING PROCESS ON PHENOLIC COMPOUNDS CONTENT OF SMOKED HERRING FILETS. APPLICATION TO IDENTIFICATION OF SMOKED FISH SAMPLE ACCORDING TO SMOKING PROCESS.

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Abstract

Smoking is a traditional fish preservation technique. It is generally agreed that phenolic compounds are the main compounds involved in preservation and organoleptic properties of smoked fish products (1). The relative concentration of phenolic compounds in these products depends on the nature of wood involved in their manufacturing (2, 3). The method of smoke generation and the smoking process (4) have a great influence on sensory characteristics of smoked salmon. The chemical features of meat smoking have been investigated (5) and the composition of dry or liquid smoke has been assessed in several studies (1, 2, 3). However, few data on the composition of phenolic compounds in smoked fish meal are available. On the other hand many methods and equipments for smoke production and smoke deposition exist but their impact on the phenolic compounds composition of fish muscle were not extensively evaluated. The impact of 5 smoking processes on ten phenolic compounds content in smoked herring fillets was evaluated. The studied compounds (phenol, p-cresol, o-cresol, guaiacol, 4-methyl guaiacol, 4-ethyl guaiacol, syringol, eugenol, 4-propyl guaiacol, isoeugenol) were previously identified as major components of smoked fish (6). The investigated processes were two traditional process using smouldering or friction smoke production, a process using liquid smoke atomisation, and two electrostatic smoking processes using smouldering or friction smoke production. For each of these processes the effects of several parameters as time smoking, smokehouse temperature, voltage or fish fillet temperature before smoking were estimated. Phenolic compounds were extracted by Simultaneous Steam Distillation- Solvent extraction and analysed by gas chromatography**.** The results of multifactor ANOVA shown that the phenolic compounds content is strongly affected by process and parameters of process. However, if the phenolic compounds percentages in flesh fish are affected by process, they are constant for a given process whatever the used parameters. Therefore multidimensional analysis (FAD) applied to phenolic compounds composition allows to discriminate the smoked fish filet samples according to the smoking process.

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P 35. TRACEABILITY OF HAKE SPECIES IN COMMERCIAL PRODUCTS

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Abstract

The genus *Merluccius* is composed by twelve species of hakes, ten of them of outmost commercial value. The large demand of hake meat in European markets together with the diversification of its sub-products, have accelerated the decline of natural populations of hake from European fisheries. In order to cope with the market needs we attend to a massive importation of foreign hake species for the fish industry. The difficulties to identify the original hake species in processed products, either morphologically or by using proteins, makes unfeasible the traceability of hake-based products. In this study we apply a simple genetic methodology based on the intraspecific homogeneity of the ITS1-rDNA, to unambiguously identify any hake species present in commercial products.

Introduction

The traceability of fish-based products is a sought-after technology used to cope with ecumenical tasks as food safety and commercial fraud. The development of diagnostic methodologies for commercial species increases with a) the increasing number of species susceptible to be marketed under the same generic name, i.e. “hake”, and b) the diversification and manipulation of sub-products which brings about the irreversible morphological modification of the original species. Whole fish specimens are easily recognisable by inspection of morphological and meristic traits (Inada, 1981). However, the identification of processed fish meat requires the development of reliable molecular tools. The first methodological attempts consisted on biochemical techniques as high resolution chromatography HPLC (Piñeiro et al., 1997) and isoelectrofocussing IEF (Céspedes et al., 1999) both techniques used to identify row stuff from unmixed samples. Later on electrophoretic techniques as SDS-PAGE and IEF-Urea (Etienne et al., 2000) were successfully applied to determine the species present in smoked meat and fried fishes, yet it proved useless for thermically degraded foods. The alternative to identification methods based on the stability of proteins was afforded from the DNA recombinant technology. Genetic analyses on mitochondrial DNA (Carrera et al., 1999) as well as on nuclear DNA (Yu et al., 2000) were successfully adapted to the genetic diagnosis of several fish-based products, and is nowadays the major source of genetic markers to identify commercial species. The goal of this study was the application of genetic diagnosis tools recently developed for the genus *Merluccius* (Montse, 2003) to determine the presence, origin and identity of frozen hake blocks imported from a third country for human consumption.

Methodology & Results

The information accompanying the good states that we are dealing with frozen hake blocks from “Argentinian hake”, but since the cargo have passed through several steps-over, the authorities wish to certify the accuracy of its origin and labelling.

1. DNA extraction

DNA extraction was performed using a Chelex resin method (Walsh et al., 1991). One piece of tissue from each 4 hake blocks were chopped and introduced independently in 1.5 mL vials containing 300 µL of 10% Chelex and 5 µL proteinase-K. The mixture was energetically vortexed, incubated at 55º C for 60 min, and heated at 100º C during 15 min. In order to co-precipitate resin and debris, the mixture was centrifuged at 12.000 *g* for 3 min, so clean DNA was recovered from the supernatant (Fig. 1).

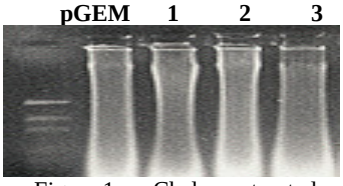

Fig. 1- Chelex-extracted DNA from four frozen hake blocks.

2. PCR Amplification of a 193 bp fragment from ITS1-rDNA

The amplification reactions were performed with the *puRe Taq Ready-to-go PCR Beads Kit* (Amersham Biosciences). The PCR beads were mixed with 5 µL of DNA, 20 pmol of primers RP1 and RP2 (Montse, 2003) and 16 µL of H_2O to adjust volumes to 25 µL. A 2% agarose gel (1X TBE) containing 1.3 µL of Ethidium Bromide (10 mg/mL), was used to electrophoresize 15 µL of the amplification product. The UV gel exposure after migration showed an amplified fragment of 193 bp in all the four samples (Fig. 2).

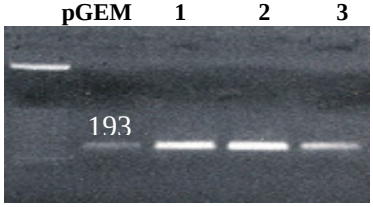

Fig. 2- PCR-amplification of a 193 bp fragment from "hake blocks".

3. PCR amplification of a 602-659 bp fragment from the ITS1-rDNA

The amplification reactions were performed with the *puRe Taq Ready-to-go PCR Beads Kit* (Amersham Biosciences). PCR beads were mixed with 5 µL of extracted DNA, 20 pmol of primers PP1 and PP2 (Montse, 2003), 1.5 mM $MgCl_2$ and 16 µL of H_2O to adjust volume to 25 µL. A 2% agarose gel (1X TBE) containing 1.3 µL of Ethidium Bromide (10 mg/mL), was used to electrophoresize 5 µL the amplification product. The UV gel exposure after migration showed an amplified fragment of 602-659 bp in all the four samples (Fig. 3).

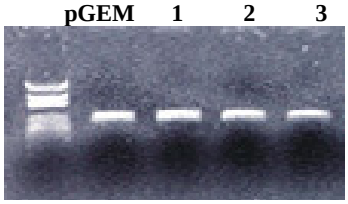

Fig. 3- PCR amplificación of a 602-659 bp fragment from "hake blocks".

4. Band purification and enzymatic digestion using ITSER1

In order to avoid interpretative artefacts likely to appear after the enzymatic digestion of the DNA, the PCR products were purified from the gel using the *GFX PCR DNA and Gel Band Purification Kit* (Amersham Biosciences). An aliquote of the purified fragment from each sample was digested with the Class II restrictase ITSER1. The reaction consisted on a mixture of 5 µL of DNA, 2 µL of enzyme buffer, 2.5 U of the enzyme and water to adjust to 20 µL. The reactions were incubated for 5 h at 37º C.

5. Restriction pattern

A 3% agarose gel (2 : 1, NuSieve: Seakem LE; 1X TBE) containing 1.3 µL of Ethidium Bromide (10 mg/mL), was used to electrophoresize 20 µL of the digestion product mixed with 3 µL of loading buffer. The molecular sizer pGEM (Promega) was co-migrated in the first column and the electrophoresis was allowed for 60 min at 70 v. The UV gel exposure after migration showed a four-banded pattern of 254, 158, 125, and 65 bp fragments in all the four samples (Fig. 4).

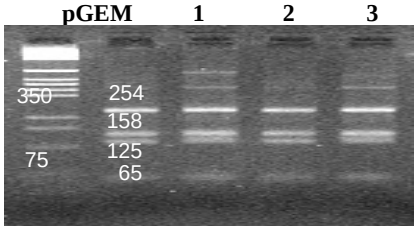

Fig. 4- Band pattern obtained with restriction enzyme ITSER1 on amplified ITS1-rDNA from four hake blocks.

Discussion

The first PCR amplification test performed to amplify a 193 bp fragment from ITS1-rDNA (Step 2) gave us evidences that the frozen blocks came in fact from a hake species. This first assessment is strongly recommended before we afford the diagnostic identification of species. This is so for an evident reason, it is a waste to try to determine which hake species is there in our sample if there were no hake in it. The second PCR amplification performed to amplify a 602-659 bp fragment (Step 3) served us to obtain the specific restriction pattern of the questioned hake species, using the diagnostic enzyme ITSER1. This restriction enzyme allowed the unambiguous identification of the species as *Merluccius hubbsi*, which restricted ITS1-rDNA presents a unique four-banded pattern for this enzyme (Fig. 4). The Official Inspectors in charge of controlling the authenticity of this imported sample would have verified that the Argentinian hake declared in the label and accompanying description was indeed *M. hubbsi*. Therefore there is no commercial fraud in this case and the taxes rendered by importers fit to legality.

We took advantage of the information accompanying the sample to focalise the diagnosis. Therefore this information prompted us to choose the restriction enzyme ITSER1 for it unambiguously identify most South-American hakes. The whole diagnostic procedure was performed in a single working day. This is an important step only provided in a few genetic assays (Cabado et al., 2003), and allows a quick decision making process on commercial goods.

Acknowledgements

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P 36. GENETIC IDENTIFICATION OF HAKE SPECIES, *Merluccius* spp., BY PCR-RFLPs ON CYTOCHROME *b* OF MITOCONDRIAL DNA

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Abstract

The application of molecular genetics to the identification of commercial fishes is one of the most sought-after technologies demanded by administrators of quality control and commercial imports. In this work we have used the genetic variation of a 465 bp from the mitochondrial DNA Cytochrome *b* gene to determine the identity of commercialised hake species. The combination of three restriction enzymes sequentially applied to that fragment allowed the full diagnosis of the species present in the sample. This genetic technology would be of great help in 1) the traceability of hakes across the food-chain, 2) the control of authenticity of origin of hake imports, 3) the control of fraud to consumers, 4) the industrial advise regarding the labelling of commercial products, and 5) the forensic genetics of hake fisheries.

Introduction

Identification of fish species of commercial importance is a legal requisite for the appropriate labelling of products, not only for consumers, but also for fish industry that buys raw fish to use in the manufacture of different products (Quinteiro et al., 1998). Protein analysis cannot be systematically applied as biochemical markers since proteins denature when food is subjected to thermal treatment (Etienne et al., 2000; Piñeiro et al., 2001). However, molecular biology techniques allow a fine analysis of DNA to set up diagnostic tools for ecumenical tasks (Carrera et al., 1999; Céspedes et al., 1999). Therefore PCR-RFLPs methodology is used successfully as a reliable way to differentiate species in raw and processed fish. The genus *Merluccius* consists of twelve species distributed along the Atlantic Ocean and the Eastern Pacific. All of them show a close morphology that is only distinguishable by experts (Inada, 1981). Since the meat quality differs among species, which in turn results in significant differences in their market prices, it is necessary to ensure product authenticity. In this study we applied a mitochondrial DNA-based method as an example of its utility and discriminatory power to identify the exact hake species present in a commercial product.

Methodology & results

We are dealing with an ultra-frozen product showing bad signals of conservation, which supposedly broke the cold chain. The information written in its label identify it as “hake medallions” from Cape hake (*Merluccius capensis* and *Merluccius paradoxus*). Sanitary authorities wish to verify the real hake species in that product to retrace its origin and market history, and adopt the suitable measures to cope with the problem.

1. DNA extraction

DNA extraction was performed using a Chelex resin method (Walsh et al. 1991). One piece of tissue from each 4 medallions were chopped and introduced in independent 1.5 mL vials containing 300 µL of 10% Chelex and 5 µL proteinase-K. The mixture was briefly vortexed, incubated at 55º C for 60 min, and heated at 100º C for 15 min. In order to pellet together resin and debris, the mixture was centrifuged at 12,000 *g* for 3 min, and the supernatant containing the clean DNA was kept for analysis. In order to verify the quantity and quality of the DNA, an aliquote of the supernatant was electrophoresed at 70 v for 30 min in a 0.8% agarose gel.

2. PCR amplification of a 122 bp fragment from the Citochrome *b* gene

The amplification reactions were performed with the *puRe Taq Ready-to-go PCR Beads Kit* (Amersham Biosciences). The PCR beads were mixed with 2 µL of DNA, 2 mM of $MgCl_2$, 20 pmol of the primers MP1 and MP2 (Montse, 2003) and 15.75 of H_2O to adjust volume to 25 µL. The PCR reaction was performed as follows: 95º C x 5 min, 30 cycles of 95º C x 1 min, 59º C x 1 min and 72º C x 1 min, followed by an extension step at 72º C for 10 min. A 2% agarose gel containing 1.3 µL Ethidium Bromide at 10 mg/mL was used to migrate 10 µL of the amplification product. The UV gel exposure after migration showed a common amplified 122 bp fragment in all the four samples (Fig. 1A).

3. PCR amplification of a 465 bp fragment from the Citochrome *b* gene

The amplification reactions were performed with the *puRe Taq Ready-to-go PCR Beads Kit* (Amersham Biosciences). The PCR beads were mixed with 2 µL of extracted DNA, 20 pmol of primers H15149AD and L14735 (Burgener, 1997), 2.0 mM $MgCl_2$ and H_2O to adjust volume to 25 µL. The PCR reaction was performed as follows: 96º C x 3 min, 40 cycles of 96º C x 30 s, 63º C x 1 min and 72º C x 1 min, followed by an extension step at 72º C x 10 min. A 2% agarose gel containing 1.3 µL Ethidium Bromide (10 mg/mL) was used to migrate 5 µL of the amplification product. The UV gel exposure after migration showed an amplified fragment of 465 bp in all the four samples (Fig. 1B).

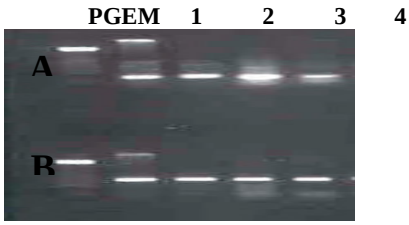

Fig. 1. PCR amplifications on the Citochrome *b* gene from four hake medallions, A) 122 bp fragments, B) 465 bp fragments.

4. Purification and digestion of the amplified fragment

In order to avoid interpretative artefacts likely to appear after DNA digestion, the PCR products were purified from the gel using the *GFX PCR DNA and Gel Band Purification Kit* (Amersham Biosciences). An aliquote of the purified fragment from each sample was digested with the Class II restrictases CITER1, CITER2 and CITER3. The reaction was performed in 1.5 mL vials and consisted on a mixture of 5 µl of purified fragment, 2 µl of enzyme buffer, 2.5 U of the enzyme and water to complete 20 µl. The reactions were incubated for 3 hours at 37º C.

5. RESTRICTION PATTERNS

A 3% agarose gel (2 : 1, NuSieve: Seakem LE) containing 1.3 µl Ethidium Bromide (10 mg/mL) was used to electrophoresize 20 µl of the digestion product mixed with 3 µl of loading buffer. The molecular sizer PGEM (Promega) was co-migrated in the first column and the electrophoresis was allowed for 60 min at 70 v. The UV gel exposure after migration showed a 465 bp fragment that was not digested with the enzyme CITER1 (Columns 1, 4 and 7), the enzyme CITER2 rendered ten sub-fragments of 165, 105, 56, 43, 32, 27, 13, 9, 9, and 6 bp (Columns 2, 5 and 8, only labelled the three largest fragments), and the enzyme CITER3 gave three sub-fragments of 237, 153, and 75 bp (Columns 3, 6 and 9) (Fig. 2).

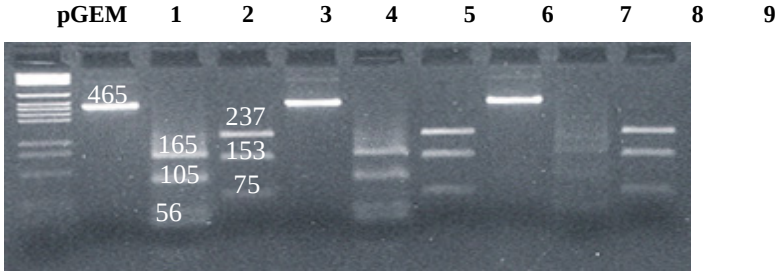

Fig. 2- Restriction patterns after the digestion of Citochrome *b* with the diagnostic enzymes CITER1, CITER2 and CITER3.

Discussion

The PCR amplification performed to amplify a 122 bp fragment from the Citochrome *b* gene (Step 2) confirmed that the marketed medallions were in fact from hake or cod species. This preliminary diagnostic test provides evidence for the presence or absence of either hake or cod in a blind sample. Therefore we would not went ahead with the whole species test in case we had got a negative result from this preliminary test. The second PCR amplification performed to amplify a 465 bp fragment (Step 3) gave us the specific restriction patterns of the questioned hake species, using the three diagnostic enzymes CITER1, CITER2 and CITER3. The 465 bp fragment that was not digested with the enzyme CITER1 was enough to classify all samples as *Merluccius capensis.* The restriction patterns obtained with the enzymes CITER2 and CITER3 simply confirmed this diagnosis.

The Sanitary Inspectors in charge of tracking the authenticity and origin of this sample would have verified that the "Cape hake" stamped in the label was indeed *M. capensis*. This genetic information would serve as a reliable tag to track back the distribution pathway followed by this altered good and help to remove it from the food chain. The whole diagnostic procedure was performed in a single working day so it allows a quick response in case of alimentary alarms. This procedure is fast, easy and cheap, and allows the identification of hake-based products with a 99.99% reliability. The present study tried to prove the diagnostic power of the methodology employed (Montse, 2003) in a case example, yet it is applicable to any fresh, frozen or cooked product so far commercialised. Using only four primer pairs and three restriction enzymes this protocol allows a) to detect the presence or absence of hake and cod in any blind sample, and b) to identify all world hake species (12) described in the literature. These properties make this DNA-based method, together with the one optimised for ITS1-rDNA (Montse et al., 2003) the fastest and cheapest procedures so far available for traceability in the genus *Merluccius* spp.

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P 37. F.I.N.S TECHNIQUE TO IDENTIFY SPECIES OF CANNED SARDINE AND SARDINE TYPE PRODUCT.

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Abstract

The EU regulation stipulated that the canned sardines must be exclusively prepared from *Sardina pilchardus* (Walbaum, 1792). However, this regulation is inconsistent with the TBT (1) Agreement and Codex Stan 94 which defines a positive list of small pelagic fish (21 species) that can be used in the preparation of canned sardine or sardine type products. EU has to modify its regulation, so identification of species in sardine type products is becoming increasingly important in Europe.

A previous phylogenetic study (2) showed that the mitochondrial DNA gene encoding cytochrome *b* allows the discrimination of closely-related species belonging to *Clupeomorpha*. The analysis of the relationships among the considered species on the complete cytochrome *b* shown that sequence analysis had the power to authenticate these related species.

A diagnostic fragment of only approximately 150 bp was amplified by PCR on canned fish because heat-sterilized process strongly hydrolyses DNA. This short fragment was double strand sequenced. Forensically Informative Nucleotide Sequencing (FINS) technique was employed for identification of fifty canned sardine and sardine type products. Multiple alignment of a partial sequence of 103 bp from the analysed reference samples and canned samples was performed and genetic distances were estimated by the Tamura and Nei (3) method. Among the fifty canned samples, half the samples was identified as sardines. The intraspecific variability observed for canned sardine was very low (< 0.02) whereas the interspecific variability calculated was superior to 0.20 for the Clupeidae species tested. A neighbor-joining tree inferred from the Tamura and Nei distances between the cytochrome *b* sequences from the numerous specimens and canned samples was obtained. Bootstrap analysis yielded strong support for clades associated with each of species references (Bootstrap 92-100%). All the individuals of commercial canned sardine and the *Sardina pilchardus* reference were grouped in the same cluster with a bootstrap value of 100%.

According to this methodology, most of the commercial canned samples tested were unequivocally identified. Nevertheless in some cases, the lack of reference species did not permit us to assign the unknown canned samples but no confused relation with the *Sardina pilchardus* clade was recorded.

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P 38. IDENTIFICATION OF TUNA AND GADOID SPECIES IN SEAFOOD PRODUCTS USING PCR-ELISA

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Abstract

This project is aimed to advance a step further in the field of species identification, using molecular techniques, by developing a diagnostic kit for the identification of fish species belonging to two groups with high commercial value: Tunas and Gadoids.

Mitochondrial DNA sequences, belonging to a wide range of species in both groups, have been analysed. Using these sequences specific mitochondrial DNA nucleotides have been searched and DNA fragments have been selected for designing capture probes in order to use them in colorimetric hybridization protocols. Also, a relational database with the collected and obtained sequences has been built up. This will be a tool for identifying species in seafood products.

The development of a commercial diagnostic kit, which is the ultimate objective of the present project, will involve a validation exercise with external laboratories.

Introduction

In order to avoid possible fraud in the labelling of seafood, the identification of fish species is becoming a topic of growing concern. Authentication of these food products becomes an unsolvable problem when the external morphological characteristics of the fish are removed during filleting or processing.

To date, several genetic techniques have been developed to authenticate seafood products. These techniques are based on the polymorphism analysis of different genetic markers amplified by the polymerase chain reaction (PCR) (Mackie *et al*., 1999; Rehbein *et al*., 1997). Although these tools are very useful, it is already necessary to analyse the genetic polymorphism to get a correct identification that involves time consuming, and certainly technically demanding.

An innovative technology consisting on PCR-Reverse cross-blot hybridization developed in biomedical area, is being used to identify pathogens as a routine assay (Sanguinetti *et al*., 1998). This technique is able to distinguish a single nucleotide polymorphism (SNP) of an amplicon. Moreover, these assays are more rapid and cheaper than others and even are capable of monitoring, that will be of great benefit for both the food industry and the governmental food control laboratories.

This project is aimed to advance a step further in the field of species identification of seafood, using innovative molecular techniques, by developing a diagnostic kit for the identification of fish species belonging to two groups with high commercial value: Tunas and Gadoids.

Methodology

Specimens of tuna and gadoid species were obtained from markets. The DNA extraction method used is a modification of that reported by Rehbein *et al.* (1997). The amplification of the mitochondrial cytochrome *b* gene was developed with primers previously described and others developed for this project. The DNA sequencing was carried out directly on the purified PCR fragments with a 3700 DNA Analyzer ABI PRISM, using the ABI Prism BigDye Terminator Cycle Sequencing Ready Reaction Kit, version 3.0 (Applied Biosystems, Foster City, USA). The multiple alignment was carried out using the Clustal W program (http://www.ebi.ac.uk/clustalw). PCR fragments were labeled with digoxigenin and tested with biotinylated probes inmovilizated to streptavidin coated microplates. Presence of digoxigenin labeled fragments was visualized with peroxidase-conjugated anti-digoxigenin antibody and a color development substrate.

Results

Collecting of tuna and gadoid species.

In order to develop this study, there have been collected 245 individuals of 34 species of tunas and gadoids around the world: *Auxis rochei, A. thazard, Boreagadus saida, Brosme brosme, Coryphaenoides rupestris, Eleginus navaga, Euthynnus alleteratus. Gadus aeglefinus, G. macrocephalus, G. mediterraneus, G. morhua, G. ogac, Gaidropsarus ensis, Katsuwonus pelamis, Merlangius merlangus, Micromesistius poutasou, Molva dypterygia, M. molva, Pollachius pollachius, P. virens, Sarda sarda, Scomber cavalla, S. chiliensis, S. japonicus,*

S. scombrus, Theragra chalcogramma, Thunnus alalunga, T. albacares, T. atlanticus, T. obesus, T. thynnus orientalis, T. thynnus thynnus, Trisopterus luscus.

Sequencing and compiling of mitochondrial DNA sequences

Analysis of mitochondrial genome (mainly of control regions, cytochrome *b* and ATPase genes) has been extensively used to resolve evolutionary relationships among close related species of the *Thunnus* genus and other fish (Kocher *et al*., 1989; Quinteiro *et al*., 1998).

In this study, fragments of mitochondrial cytochrome *b* gene belonging to tuna species (denominated BDR and BMID) have been sequenced after their amplification with those primers designed by Quinteiro *et al*. (1998). On the other hand, several primers have been designed to amplify the whole of the citochrome *b* gene of those species of gadoids and tunas more valuable for the food industry.

All mitochondrial sequences obtained in this work and those compiled from GenBank were saved onto a relational database (480 sequences). This database will be accessible via internet after implementation with the interface that Active Server Pages (ASPs) supplies.

Selection of DNA fragments to design capture probes.

To date, five biotinylated capture probes enclosed with BDR and BMID fragments have been obtained and tested in streptavidin coated microplates. Four of these probes correspond with the identification of *T. alalunga*, *T. albacares*, *K. pelamis* and *Thunnus* spp. and the last one is specific for the previous species and for *A. rocheii*, *S. sarda*, *T. obesus* and *T. thynnus*.

Concerning gadoids, five capture probes have been designed. Preliminary studies indicate that they will allow the differentiation between *G. morhua*, *B. brosme*, *Molva* spp., *Pollachius* spp. and *T. chalcogramma*.

These are the preliminary results of a study that will be continued with the optimisation of DNA extraction methods from different types of seafood products together with the development of a commercial diagnostic kit for gadoids and tunas, which is the ultimate objective of the present project. This will involve a validation exercise with external laboratories to confirm the usefulness of the developed techniques and their implementation in routine laboratory practices.

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P 39. ATLANTIC COD TRYPSIN I PRODUCED BY BIOTECHNOLOGY METHODS

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Abstract

Atlantic cod trypsin I isolated from its native source has already proven its usefulness in various industrial applications. In order to facilitate the production of this enzyme by expression in a microorganism a cDNA clone corresponding to trypsin I was isolated from an Atlantic cod pyloric cacea cDNA library and sequenced. Trypsin I is the most abundant and best characterised form of seven trypsin isoenzymes that have been isolated from the pyloric cacea of Atlantic cod (*Gadus morhua*) and purified. Also, trypsin I shows the highest catalytic efficiency of these isoforms. The superior catalytic efficiency of Atlantic cod trypsin I makes it a very attractive enzyme to study with respect to structure and function. The purpose of the research project was to express trypsin I-K (K=cloned) from Atlantic cod and confirm that it is identical to trypsin I-N (N=native).

The precursor form of trypsin I-K was produced in a new *E.coli* His-Patch Thio-Fusion expression system. This enables purification of the expressed enzyme on a metal binding ProBond affinity column. The trypsin I-K cDNA was cloned into the pThioHis expression vector such that the HP-thioredoxin part of the vector is fused to the 5´-end of the gene. The Thio part of the HP-thioredoxin-trypsin I-K fusion protein increases its solubility in addition to binding to the ProBond affinity column. After purification on ProBond, the active mature part of trypsin I-K is cleaved from the fusion protein with a small amount of cod trypsin. Trypsin I-K was further purified on a trypsin specific *p*-aminobenzamidine affinity column. The activation step of trypsin is very difficult due to the sensitivity of the psychrophilic trypsin I towards inactivation by heat and autolysis. The results show that trypsin I-K and trypsin I-N are most likely the same enzyme.

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P 40. THE NOVEL TRYPSIN Y FROM ATLANTIC COD – CLONING, EXPRESSION AND PURIFICATION.

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Abstract

Trypsins and other serine proteases from fish have many potential industrial applications. Trypsins from the Atlantic cod are already being used in cosmetic products as well as to produce flavourants from seafood. In our search for serine proteases with new characteristics we have recently isolated a cDNA encoding a novel trypsin, termed trypsin Y, from an Atlantic cod cDNA library (Spilliaert and Gudmundsdóttir, 1999).

Trypsin Y, along with seven other fish trypsins, belongs to a novel clade of trypsins, the group III (Spilliaert and Gudmundsdóttir, 1999; Roach, 2002). Today, no biochemical data is available on any of the group III enzymes to supplement their molecular description, as none of these enzymes have been isolated from their natural sources. The group III trypsins have been suggested to be extreme psychrophilic enzymes (Roach, 2002). The cDNAs for the group III trypsins have been isolated from fish spending at least some part of their lives at temperature near 0°C.

The trypsinogen Y cDNA was cloned into two different expression vectors: pPICZαA *Pichia pastoris* vector and pThioHisA *E.coli* vector. The recombinant trypsin Y polypeptide was produced in both the expression systems and purified by ion exchange and affinity chromatography. The thermal stability and the activity of the recombinant trypsin Y were measured towards synthetic substrates for trypsin, chymotrypsin and elastase. Polyclonal antibodies were produced from the recombinant trypsin Y protein by immunizing a rabbit with a fully purified enzyme.

Our preliminary data indicate that trypsin Y is an extreme psychrophilic enzyme which is active at subzero temperatures. This enzyme seems to have broader substrate specificity than trypsin I previously isolated from the Atlantic cod. Our future research will focus on the search for the native trypsin Y in the Atlantic cod using the polyclonal antibody made from the recombinant trypsin Y.

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P 41. CATHEPSIN ACTIVITY IN HERRING (*CLUPEA HARENGUS* L.) CAUGHT AT DIFFERENT LOCATIONS AND DIFFERENT SEASONS

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Abstract

Herring (*Clupea harengus* L.) is by weight the most important fish species for consumption in Denmark. Since the herring population structure is rather complex, a given catch may consist of herring with different biological characteristics, which may result in different functional and sensory properties. In order to optimise the resource utilisation of herring a thorough study of the correlations between sensory, biological, functional and biochemical characteristics is necessary.

Post-mortem proteolysis could be an important biochemical parameter, which may influence the functional and sensory properties of herring as a raw material for the fish industry. Cathepsins are proteolytic enzymes that have been demonstrated to have a high activity at low *post-mortem* pH. The object of the present study is to implement specific enzyme assays for cathepsin B and cathepsin L and to measure the activities in herring caught at different seasons (year 2001 and 2002) in the North Sea, Skagerrak, Kattegat and in the Baltic Sea (Figure 1). At the present time the correlations between enzyme activities, biological characteristics and catch season and catch location are studied (Figure 2). Future investigations will also include sensory properties and other biochemical parameters such as lipid content and oxidation in the data analysis.

Specific activity of cathepsin L and cathepsin B are measured in herring muscle homogenates with specific fluorogenic substrates: Z-Arg-Arg-MCA for cathepsin B and Z-Phe-Arg-MCA for cathepsin L. Information about biological factors such as length, weight, age, sex, maturity and spawning type are available. The enzyme activity data and biological data are analysed using the multivariate method: principal component analysis (PCA). The PCA analysis clearly showed that the cathepsin activities in herring from different areas were mainly overlapping, however there was a tendency for cathepsin B and cathepsin L activity to be higher in herring caught in Skagerrak than herring caught in the Baltic Sea. The Baltic Sea herring is caught in the winter and autumn periods, whereas the herring from Skagerrak was caught in the spring and summer periods. That means that the effect of fishing ground and season are partly confounded. At the present time there was not found any correlations between cathepsin activities and biological parameters, such as size, sex maturity and spawning type. Further investigation will reveal if there are any correlations between cathepsin activities and genetic measurements as well as sensory properties.

Cruise #	Year	Month	Water
1	2001	July	North Sea Skagerrak Kattegat
2		August	North Sea
3		November	Baltic Sea
4	2002	March	Baltic Sea
5		June	North Sea
		July	North Sea Skagerrak Kattegat

Figure 1. Experimental design. All 400 herring used in the present experiment are derived from five cruises distributed over the years 2001 and 2002. The cruises took place in five different months (catch seasons) and four different waters (catch locations) as shown in the figure.

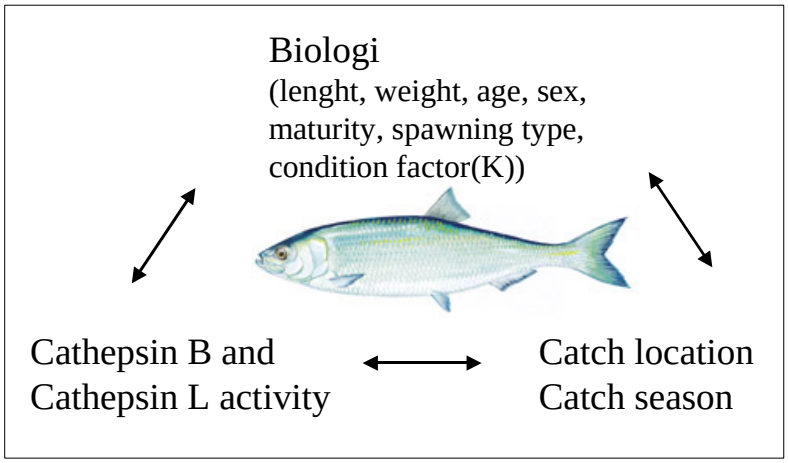

Figure 2. The objective of the present communication is to measure the activity of cathepsin B and cathepsin L in crude extracts from herring and to look for correlations between enzyme activities, biological variables and catch location and season.

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P 42. PARTIAL PURIFICATION AND BIOCHEMICAL CHARACTERIZATION OF TMAOASE FROM KIDNEY OF EUROPEAN HAKE (*MERLUCCIUS MERLUCCIUS*)

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Abstract

The trimethylamine N-oxide aldolase (TMAOase) activity from kidney of European hake (*Merluccius merluccius*) had been characterised by using chromatographic and electrophoretic techniques.

Soluble TMAOase was obtained using a differential centrifugation protocol with CHAPS and NaCl buffer. Two TMAOase fractions were isolated by ionic exchange chromatography using a FPLC system. For this purpose the weak anion exchanger ANX Sepharose 4 Fast Flow (high sub), suited for high molecular-mass proteins, was employed. After AE chromatography, fractions were separated by native electrophoresis. One of the fractions eluted at 0.45 M sodium chloride, and have a charge/mass relation in electrophoresis similar to bovine serum albumin, with a molecular weight over 30 kDa. The other one eluted at 0.7 M sodium chloride, and presented a very low electrophoretic mobility due either to a low charge/mass relation, or the presence of protein aggregates of different sizes between 440–2,000 kDa.

Proteins present in both TMAOase fractions showed acid isoelectric points between 4.55-5.85 pH values.

Introduction

During frozen storage of gadoid species, formaldehyde (FA) produced by trimethylamine N-oxide aldolase (TMAOase) causes a very important quality loss of these fish (Babbit and col., 1972; Castell and col., 1973; Matthews and col., 1980; Parkin and Hultin, 1982). This fact has carried that various authors has to try to purify and to characterize this enzyme, concluding that it is a multienzymatic system.

Gill and Paulson (1982), in their work about TMAOase from kidney of cod (*Gadus morhua*), isolated four isozymes with different isoelectric points (pI) located at acid pH values. Reece (1983) described the presence of two types of enzymes with TMAOase activity present in cod muscle. One of them was inhibited in the presence of oxygen and potassium cyanide, and the other one was not affected by any of these two factors. Parkin and Hultin (1986b) isolated a fraction with TMAOase activity from the microsomal fraction of red hake muscle (*Urophycis chuss*), which was composed by various protein components. Two cofactor system were capable of stimulating this enzymatic activity. One of them would utilize the cofactors system NADH/FMN, and would require anaerobic conditions, while another one would require iron and ascorbate and/or cysteine, independently of the presence or absence of oxygen (Parkin and Hultin, 1986a). Joly and col. (1992) isolated from saithe kidney (*Pollachius virens*) three high molecular weight independent enzymes (200-2,000 kDa) by anionic exchange chromatography. Although their isoelectric points were different (4.1, 4.5 and 5.0), all of them showed the same optimal pH (pH 5,1), the same affinity constant (Km 12 mM), and the same activation energy (4 kcal mol-1). Also, these authors described the presence of micelles of phospholipids in these high molecular weight protein components. Havemeister in her study about purification and biochemical characterization of TMAOase activity of whiting (*Merlangius merlangus*) found both TMAOase soluble and membrane bound, being kidney the organs with the highest level of this enzymatic activity. Two fractions of TMAOase were isolated; one of them of high molecular weight (2,000 KDa) was a membrane protein, and another one of low molecular weight (150-600 KDa) was soluble. Both fractions shown be heat stable up to 30°- 40°C for 20 min. Maximum activity of partially purified TMAOase was at 25°C and pH 4.5 - 5.0. Its activation energy was 5.2-6.0 Kcal/mol °K, and the apparent Km for its substrate TMAO was found between 5.7 – 11.5 mM. Kimura and col. (2000a,b) partially isolated TMAOase present in muscle of Alaska pollock (*Theragra chalcogramma*) by using (DEAE)-cellulose and gel-filtration. These authors concluded that optimum pH values for this enzymatic activity in a crude extract of flesh were 7.0 – 7.5, while optimum pH for partially purified TMAOase was 7.0. No activity was detected above 30°C, Km for TMAO was around 30 mM, with an activation energy of 38.4 Kj/mol °C. Finally, the molecular weight for this enzyme was around 400 KDa.

The lack of study about purification and characterization of this enzymatic activity in European hake (*Merluccius merluccius*), high commercial value species, has originated this work.

Materials and Methods

A soluble TMAOase extract (S3) was made from kidney of European hake using CHAPS to release the enzyme from subcellular membranes. The protocol using during extraction is shown in the figure 1.

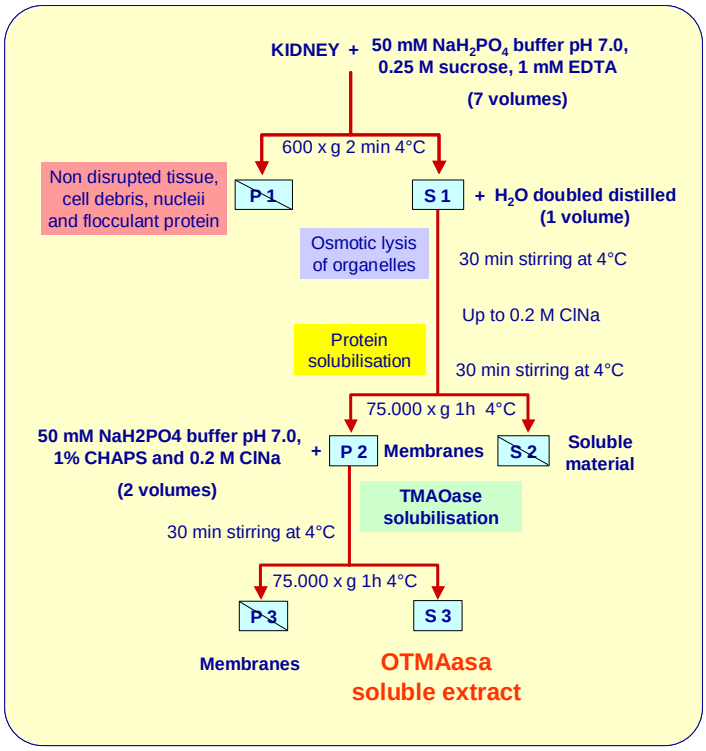

Figure 1. Differential centrifugation protocol employed to obtain a soluble TMAOase extract from kidney of European hake.

The fractionation of the proteins of supernatant S3 was done by ionic exchange chromatography using a XK 16/20 column (Amersham Pharmacia Biotech, Uppsala, Sweden) containing the weak anion exchanger ANX Sepharose 4 Fast Flow (High sub) (Amersham Pharmacia Biotech) on a FPLC system (Fast Performance Liquid Chromatography, Amersham Pharmacia Biotech).

For isoelectric focusing (IEF) commercial dried polyacrylamide gels “Cleangel IEF” T%=5, C%=3 (Amersham Pharmacia Biotech, Uppsala, Sweden) were employed. The gels were rehydrated with a solution containing 8M urea and 1% CHAPS since the most of proteins present in extract S3 were membrane proteins. After runs gels were stained with Coomassie Brilliant Blue R-250 (BIO RAD).

Native electrophoresis were made using the method 'blue native-polyacrylamide gel electrophoresis' (BN-PAGE) described by Schägger and von Jagow, 1994.

Gels of different polyacrylamide gradients (T%=5-13, T%=4-8, and T%=4-20) of 18 x 16 cm (width x length) were prepared and onto these resolving gels stacking gels T%=4 or T%=5 were polymerized. After runs, the gels were cut in half (Figure 2). One half was stained for TMAOase activity. For this purpose, first the gels were incubated in a solution containing substrate and cofactors of enzyme (Nielsen and col. ,2000) for 10 min. After that, concentrated Nash reagent (x4) was loaded onto the gels and were incubated at 40°C until yellow color appeared in zones of gel where FA had been produced by TMAOase (Nash, 1953).

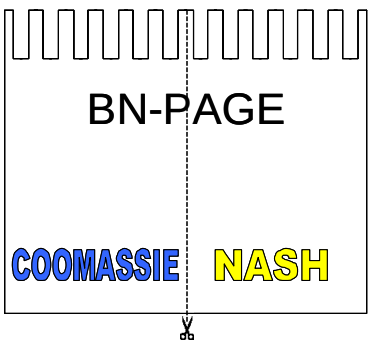

Figure 2. Stain for electrophoresis BN-PAGE

Protein were detected by staining the other half of the gel with Coomassie Brilliant Blue (Schägger, 1984).

Samples employed both for chromatography and electrophoresis were previously desalted on PD-10 columns (Amersham Pharmacia Biotech).

TMAOase activity was measured using a two-step assay using the enzyme formaldehyde dehydrogenase described by Nielsen and others (2000).

Results

Two TMAOase fractions were isolated from TMAOase soluble extract S3 by ionic exchange chromatography (Figure 3). The first one (TMAOase fraction 1) eluted at 0.45 M sodium chloride , while the second one (TMAOase fraction 2) eluted at 0.70 M sodium chloride.

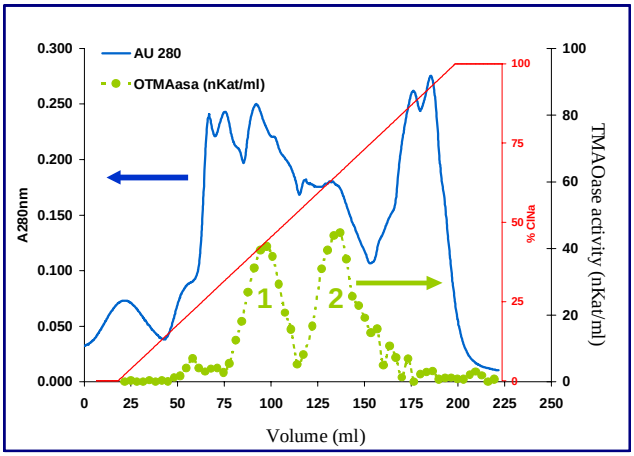

Figure 3. Ionic exchange chromatography of soluble TMAOase extract S3.

As it can be seen in figure 4, proteins present in both TMAOase fractions showed acid isoelectric points between 4.55 - 5.85 pH values. However, in spite of the inclusion in the matrix of gel of substances like CHAPS and urea for increasing the solubility of membrane bound proteins , it was not possible to keep soluble all proteins present in analysed fractions and protein precipitation was observed in the upper portion of gel where the extract S3 and TMAOase fraction 2 were loaded.

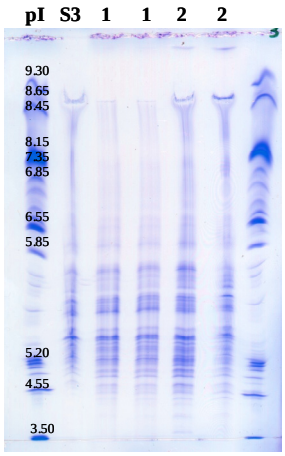

Figure 4. Separation by IEF of protein components present in both TMAOase fractions and soluble TMAOase extract S3.

To determine if one or several proteins are the responsible of TMAOase activity of kidney of European hake, the protein components present in both fractions were separated by native electrophoresis (BN-PAGE). This technique allows the enzyme to keep active after run and to be detected. Both TMAOase fractions were run separately in a gel with a polyacrylamide gradient T=5-13%. The soluble extract of TMAOase S3 was also loaded onto the gels. In figure 5 it can be seen the resulting gels. The separation of the TMAOase fraction 1 (gel 1) shows TMAOase activity in the lower part of the gel only (zone A), next to the location of the bovine serum albumin (BSA) used as standard. The S3 extract was also loaded in the gel, and besides showing TMAOase activity at the

same height as fraction 1, it also revealed TMAOase in the stacking gel (zone B) and in the and upper portion of resolving gel (zone C), which in the portion of the gel stained with Coomassie would correspond to precipitated protein. In order to resolve possible components of TMAOase activity from zone A of fraction 1, the polyacrylamide gel was modified up to T=4-20% (gel 3). The resulting gel (gel 3) showed again a unique zone with TMAOase activity at the same level as that of bovine serum albumin (zone A).

Regarding fraction TMAOase 2, when the polyacrylamide gradient was T=5-13%. (gel 2), activity was localized in the stacking gel (zone B), upper portion of resolving gel, but without getting into it (zone C). Sample S3 also presented activity at the level of bovine serum albumin (zone A). A BN-PAGE was carried out with a very low percentage of polyacrylamide, T=4-8%, so as to facilitate the entry of this second TMAOase fraction in the gel. In the resulting gel (gel 4) it can be seen how this decrease of polyacrylamide content allows that the enzyme of zone C can penetrate in the gel, remaining finally at an intermediate height between the standards of phosphorilase B (Fosf B) and bovine serum albumin (BSA). However, TMAOase is still detected in the stacking gel and the upper zone of the resolving gel.

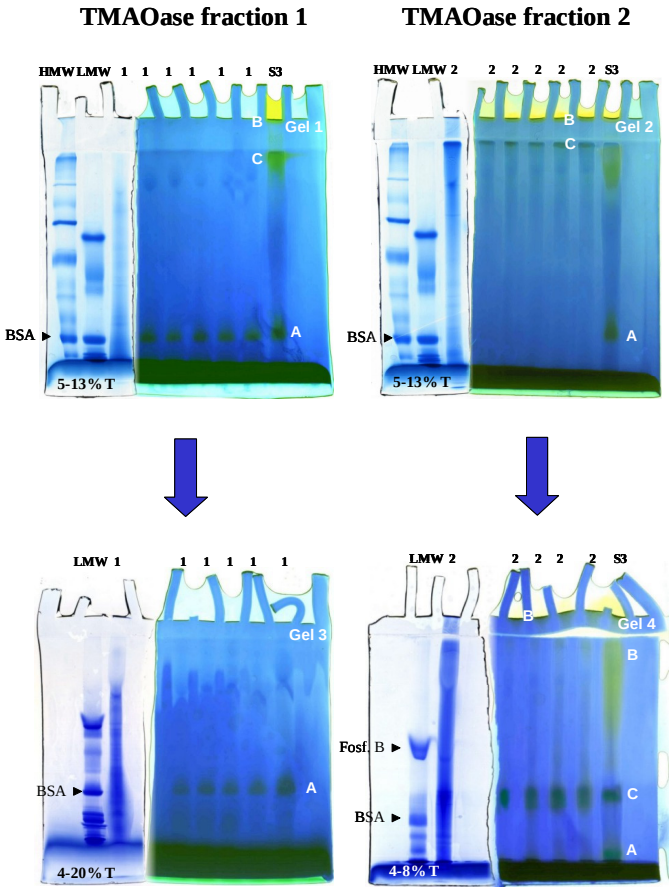

Figure 5. BN-PAGE electrophoresis of both TMAOase fractions.

Conclusions

The TMAOase activity present in kidney of European hake is associated to two protein fractions isolated by ionic exchange chromatography. The first one eluted when sodium chloride concentration was 0.45 M. Its charge/mass relation is similar to that of bovine serum albumin. The second fraction eluted when salt concentration was 0.70 M. Its charge/mass relation is very low, even it could be forming protein aggregates of high molecular weight.

It is possible that the facility of the TMAOase units to form multimer aggregates in highly hydrophilic environments could be due to its high hydrophobicity, as suggested by the difficulties found to keep it in solution.

Proteins present in these two fractions showed acid isoelectric points between 4.55 - 5.85 pH values.

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Scientific bases for international standards on safety of fish products.

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K 5. SCIENTIFIC BASIS FOR SAFETY STANDARDS FOR FISHERY PRODUCTS

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Introduction

Fish and fishery products contribute a major proportion of the total world food supply. Recent FAO data indicates world production of fish, crustaceans and molluscs reached 126.2 million tonnes in 1999, an increase of 7.2 percent above the 1998 level. The capture fisheries production, which accounted for 74 percent of the total, increased to 92.9 million tonnes, while aquaculture production increased from 30.8 to 33.3 million tonnes. The value of world total fishery production grew by 7 percent to an estimated US$ 125 billion. Fish and fishery products also contribute a major proportion of foodborne illness, ranking third on the list of products which caused foodborne disease in the United States of America between 1983 and 1992 (Lipp and Rose 1997).

Various standards and regulations, both national and international, are in place to reduce the risk of illness from consumption of fish and fishery products. Examples of such regulations include the Canadian Fish Inspection Act, the Canadian Food and Drugs Act, and the USFDA Federal Food, Drug, and Cosmetic Act. International standards for food safety are setup by the Codex Alimentarius Commission, an intergovernmental body with the purpose of implementation of the Joint FAO/WHO Food Standards Programme. A proposed Draft Code of Practice for Fish and Fishery Products is currently under review by Codex. This paper summarizes the microbiological hazards associated with fish and fishery products, and how the hazards are managed. Several specific examples of the application of regulations to management of microbiological hazards are cited.

1. Bacterial Pathogens associated with raw and processed seafood

a. *Aeromonas* species.

While mesophilic *Aeromonas* species., including *A. hydrophila*, *A. caviae*, *A. veronii* biovar *sobria*, *A. jandaei* and *A. schubertii* have been demonstrated to cause serious extraintestinal infections, *Aeromonas* species are still considered as controversial gastrointestinal pathogens (Kirov 2001). Over the past decade, *Aeromonas* species have been implicated in food-poisoning outbreaks in several countries including Japan, Norway, Sweden and France (Kirov 2001; Krovacek and others 1995). Shrimp cocktail has been implicated in an outbreak of *Aeromonas* associated gastrotenteritis (Altwegg and others 1991). *Aeromonas* species are a common cause of diseases of aquatic animals such as finfish, shellfish, crustaceans, and amphibians (Kirov 2001), and have been isolated from raw and ready-to-eat fish products (Gobat and Jemmi 1993; Gobat and Jemmi 1995; Radu and others 2003; Tsai and Chen 1996). Thermal inactivation is the primary control for *Aeromonas* species in fish and fishery products.

b. *Clostridium botulinum*

The species *Clostridium botulinum* comprises gram-positive, anaerobic, rod-shaped, spore-forming bacteria. They are distinguished from other bacteria by production of the most potent biological toxin known, botulinum neurotoxin (Prevot 1953). Strains of *C. botulinum* can be classified into seven types, A through G, based on the serological specificity of the neurotoxin produced by the strain. Human botulism is associated with types A, B, E and F, with type E being the type most often associated with illness from fishery products.

Occurrence of spores of *C. botulinum* type E in prepared fish products is common, with levels often exceeding 10^2 spores per kg (Austin and Dodds 2000). Botulism from fishery products is well documented. Thermally processed fishery products prepared both commercially and in the home have caused botulism (Armstrong and others 1969; Ball and others 1979). Four cases of botulism in Birmingham, UK, with two fatalities, were caused by canned Alaskan salmon (Gilbert and Willis 1980). This outbreak was attributed to postprocess contamination of the canned salmon through a defective can (Ball and others 1979).

Several outbreaks of botulism have been recorded from consumption of fermented fishery products. Consumption of traditionally prepared seal, whale or walrus meat, fish heads, and fish eggs in northern Canada and Alaska is a common cause of botulism (Proulx and others 1997; Shaffer and others 1990; Wainwright 1993; Wainwright and others 1988). Botulism outbreaks have alos occurred in Japan after consumption of fermented izushi (Sakaguchi 1997). The low amounts of fermentable carbohydrates in fishery products prevents a significant reduction of pH when these products are fermented. The maintenance of a neutral pH allows spores of *C. botulinum* to germinate and grow, producing botulinum neurotoxin.

Uneviscerated fish that has been salt-cured and air-dried has a history of botulism outbreaks. A massive outbreak of type E botulism, associated with traditional uneviscerated, salted fish, occurred in Cairo in April 1991 (Weber and others 1993). This was the largest reported outbreak of type E botulism, with 91 hospitalized patients.

Official reports showed that 18 of 91 (20%) reported patients died, although the toll was likely higher as coffins and mourning clothes were sold out in the area of outbreak. The outbreak was associated with eating faseikh, an uneviscerated, salted mullet fish. Very high levels of type E botulinum toxin were detected in the faseikh produced at a particular store in Cairo.

Kapchunka is prepared by salting layers of uneviscerated whitefish and curing for a minimum of 25 days. Three outbreaks of botulism from kapchunka occurred throughout the 1980's. The 1987 outbreak, caused by kapchunka commercially produced in New York City, involved eight cases, six in Israel and two in New York City. It is likely that spores of *C. botulinum* within the viscera germinated and grew in the relatively low salt "protective" environment of the viscera. As a result of these outbreaks, permanent regulations were passed prohibiting the processing, distribution, and sale of raw, uneviscerated, salt-cured fish products within New York City (Telzak and others 1990).

Commercially prepared whitefish was responsible for 22 cases, including 7 fatalities, of botulism in the United States in 1963 (Centers for Disease Control and Prevention 1998). Several factors may have contributed to these cases: the water phase NaCl concentration of the fish was likely less than 1%; the smoked products were vacuum packaged; and they were temperature abused during distribution (Eklund 1993). Commercial smoked whitefish continues to be a risk for botulism as witnessed by two recent cases of type E botulism in Germany caused by Canadian whitefish processed in Finland (Korkeala and others 1998).

The risk of botulism from fishery products is typically controlled by prevention of growth of *C. botulinum*, usually by a combination of refrigeration and adjusting the water phase salt content to greater than 3%, and storage of the product at a temperature less than 3EC for a defined shelf-life. Packaging smoked fish products in a packaging film with an oxygen permeability greater than 5,000 cc/m^2/24h allows the product to spoil before becoming toxic (Dufresne and others 2000).

c. Listeria monocytogenes

Listeria monocytogenes is a small, gram positive, rod-shaped, facultatively anaerobic bacterium capable of growth at refrigeration temperatures (Donnelly 2001). The predominant clinical forms of listeriosis are infections of the central nervous system, sepsis, abortion, and stillbirth. Gastrointestinal symptoms occur in approximately one-third of documented cases of listeriosis.

Vacuum-packaged cold-smoked rainbow trout and salmon may contain large numbers of *L. monocytogenes* and should be considered as a potential source of infection (Ericsson and others 1997; Guyer and Jemmi 1991; Johansson and others 1999; Miettinen and others 1999). Fishery products have been implicated in listeriosis cases. Raw fish and shellfish were epidemiologically linked to several cases of perinatal listeriosis in Auckland, New Zealand (Lennon and others 1984). Vacuum-packaged salmon and rainbow trout have been associated with cases of listeriosis. An outbreak of listeriosis in Sweden, consisting of nine cases occurring over a period of eleven months, was attributed to *L. monocytogenes*. At least six of the nine cases were caused by commercially produced gravad or cold-smoked rainbow trout (Ericsson and others 1997; Tham and others 2000). An additional outbreak of listeriosis, involving five patients who consumed vacuum-packaged cold-smoked rainbow trout, has been reported in Finland (Miettinen and others 1999).

The frequency of contamination of raw fish with *L. monocytogenes* is very low, with contamination occurring during processing (Autio and others 1999). *L. monocytogenes* may enter fish processing plants via seawater, utensils, staff or raw fish. Control of *L. monocytogenes* in cold-smoked fish is dependant upon the hygienic condition of the brining solution, and contact surfaces of processing equipment including slicing equipment and conveyer belts. The production process for cold smoked fish does not kill *L. monocytogenes* (Guyer and Jemmi 1991; Jemmi and Keusch 1992; Poysky and others 1997). Vacuum packaging extends the shelf-life by retarding spoilage of smoked fish products, allowing an extended time for growth of *L. monocytogenes*. A combination of strict adherence to a low storage temperature (preferably a maximum temperature of 3EC) and a limited shelf life (14 to 21 days) are recommended to control the growth of *L. monocytogenes* in these products.

d. *Salmonella*.

The genus *Salmonella* contains two species, each of which contain multiple serovars. The two species are designated as *S. enterica* and *S. bongori*. All *Salmonella* species are gram-negative bacteria belonging to the family Enterobacteriaceae. They are widely distributed and have been isolated from soil, water, foods and the intestinal tracts of humans and animals. An extensive study was completed by field laboratories of the U.S. Food and Drug Administration where 11,312 import and 768 domestic seafood samples over a 9-year period (1990 to 1998) were collected and tested for the presence of *Salmonella* (Heinitz and others 2000). Nearly 10% of import and 2.8% of domestic raw seafood were positive for *Salmonella*. *Salmonella* species were found in 2.6% of ready-to-eat imported seafood, including cooked shrimp, shellfish or fish paste, smoked fish, salted/dried fish, and caviar.

In the United States, typhoid fever was the most common shellfish borne disease (i.e., those associated with consumption of clams, oysters, mussels, and scallops) until increased surveillance and more effective sewage treatment procedures eliminated *Salmonella enterica* serovar Typhi from shellfish in the mid 1950's (Rippey 1994). Typhoid fever continues to be a problem associated with consumption of raw shellfish in some parts of the world. An outbreak of typhoid fever occurred in southwestern Ontario, Canada, after consumption of raw shellfish. In this case, fresh lapa had been purchased from different fish markets on separate dates, and the product was traced to a Toronto area wholesaler who had imported the product from Spain (Styliadis and Borczyk 1994).

Outbreaks of non-typhoidal salmonellosis have been attributed a variety of fish and shellfish products including mussels (Nakano and others 2002), dried anchovies (Ling and others 2002), smoked eel (Fell and others 2000), cockles (Greenwood and others 1998), and smoked whitefish (Gangarosa and others 1968). A large

outbreak of *Salmonella enterica* serovar Montevideo, consisting of 87 cases amongst guests attending two social functions held within 24 h, food for both having been provided by the same catering firm, was caused by improperly prepared salmon (Cartwright and Evans 1988).

Prevention of salmonellosis from consumption of fish and fishery products is achieved by reduction in contamination of products by surveillance for fecal coliforms and proper sewage treatment; and by adequate thermal processing of products to destroy *Salmonella*. Closure of shellfish beds with fecal coliform counts which exceed regulations has been successful in reducing the incidence of both typhoidal and non-typhoidal salmonellosis from shellfish.

e. *Vibrio* species

Vibrios are generally the predominant bacterial genus in estuarine waters and are assoicated with a variety of seafoods . The *Vibrio* species most often associated with illness attributed to commercial fishery products are *V. parahaemolyticus* and *V. vulnificus.* Both organisms are considered normal microflora in marine environments, and are not correlated with fecal contamination. *Vibrio parahemolyticus* has been well documented to cause outbreaks of infectious diarrhea, usually related to poor food handling; only rarely has it been reported to cause fetal septicemia (Hally and others 1995). In contrast, consumption of raw shellfish is a risk for *Vibrio vulnificus* septicemia, a severe illness with a case-fatality rate of approximately 50%. Persons most at risk include immunocompromised patients or those with liver disease (Gholami and others 1998). In California, from 1983 through July 1993, 24 cases of *V. vulnificus* infections (including 18 deaths) were reported to the Department of Health Services. In 15 of the 24 (63%) cases, a report of consumption of raw shellfish prior to illness was available (Styliadis and Borczyk 1994). Risk of illness correlates with seasonally high numbers of *V. parahaemolyticus* and *V. vulnificus* during the summer months.

During July-September 1998, an outbreak of *Vibrio parahaemolyticus* infections associated with consumption of oysters and clams harvested from Long Island Sound occurred among residents of Connecticut, New Jersey, and New York. This was an unusual outbreak, as it was the first reported outbreak of *V. parahaemolyticus* linked to consumption of shellfish harvested from the Atlantic Ocean off the state of New York (Wechsler and others 1999). During July-August 1997, the largest reported outbreak in North America of culture-confirmed *Vibrio parahaemolyticus* infections occurred along the Pacific coast of North America. Two-hundred and nine cases, with a single fatality, were associated with eating raw oysters harvested from California, Oregon, and Washington in the United States and from British Columbia in Canada. It was suggested that elevated water temperatures may have contributed to the large outbreak (Fyfe and others 1998).

Vibrio infections from seafood, unlike illnesses caused by *Salmonella* and enteric viruses, cannot be controlled by routine monitoring of shellfish beds for fecal coliforms. As a result of these large outbreaks of *V. parahaemolyticus*, the US Food and Drug Administration (FDA) advised the Interstate Shellfish Sanitation Conference (ISSC) of the need to monitor for *V. parahaemolyticus*. Oysters are now monitored for *V. parahaemolyticus* during the summer months in states where outbreaks and sporadic cases have occurred. Areas of shellfish harvesting that are found positive for *V. parahaemolyticus* are closed until the organism is no longer detected (Oliver and Ostroff 2001).

V. vulnificus and *V. parahaemolyticus* are psychrotrophic bacteria, capable of growth at temperatures as low as 4EC. Initial contamination may grow if shellfish are temperature abused during processing, transportation, retail, or in the home. Cooking of shellfish to an internal temperature of 60EC for 5 to 6 minutes is sufficient to destroy both organisms. Consumer education is also used to discourage the consumption of uncooked shellfish. The state of California has passed regulations stating that, where raw oysters are offered for sale, a sign bearing a warning stating that consumption of raw oysters from the Gulf of Mexico “may cause severe illness and even death in persons who have liver disease, cancer or other chronic illnesses that weaken the immune system” must be posted.

3. Parasites associated with raw and processed seafood

The primary parasites associated with fishery products are the nematodes *Anisakis simplex* (herring worm) and *Pseudoterranova decipians* (cod worm), and the cestode *Diphyllobothrium* species. The larval stage of *Anisakis* and related roundworms may be acquired by consumption of raw or insufficiently cooked herring, cod, Alaskan pollack, or other fish. Various marine mammals such as seals, porpoises, and whales harbor the adult worms in their intestinal tract. A non-invasive form of anisakiasis is referred to as “tingling throat syndrome” when worms “recognize” that they are not in the correct host and travel up the esophagus after release from the fish by digestion (Hayunga 2001). In the invasive form of anisakiasis, the worms penetrate the mucosa of the stomach or the small intestine, causing epigastric pain, nausea, vomiting, and diarrhea within hours after eating the seafood. The only effective treatment is surgical removal of the worms (Hayunga 2001). *P. decipians* infects seals and fish around the eastern shores of Canada. Visual inspection for these worms is performed by “candling”, where filleted fish are passed over a strong light which reveals the coiled worm silhouetted in the fish muscle.

Diphyllobothrium species. (fish tapeworm) is transmitted by consumption of raw infected fish. The adult parasite lives in the human intestinal lumen and may cause abdominal pains or a rare vitamin B_{12} deficiency. Parasites in fish are typically controlled by freezing [for at least 24 hours at -20°C], heating, or a combination of salt content and storage time. The U. S. Food and Drug Administration recommends that all fish and shellfish intended for consumption without a prior cooking process be blast frozen to -35EC or below for 15 hours or frozen to -20EC or below for seven days (Hayunga 2001).

4. Viruses that sometimes contaminate raw seafood

The two viruses of most concern in raw seafood products are norovirus (formally Norwalk virus) and

hepatitis A virus (HAV). Norovirus and HAV are spread through several modes of transmission: direct person-to-person contact, consumption of contaminated food or water, airborne droplets of vomitus, and contact with contaminated environmental surfaces. The infectious dose of norovirus is as little as 10 viral particles (Anderson and others 2003; Koopmans and others 2002). While contamination of any food may occur by contact with an infected food handler, certain foods are implicated more often in virus outbreaks. Consumption of contaminated uncooked shellfish has been responsible for several outbreaks of both norovirus and HAV (Centers for Disease Control 1995; Chalmers and McMillan 1995; Rippey 1994; Romalde and others 2001; Sanchez and others 2002; Shieh and others 2000; Stafford and others 1997). To reduce the risk enteric viral illness from consumption of shellfish, indicators are used to detect fecal contamination of shellfish beds. The European Community (EC; directive 91/492) has pronounced *Escherichia coli* an indicator organism for fecal contamination in shellfish.

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L 41. THE EFFECTS OF MODIFIED ATMOSPHERE PACKAGING ON TOXIN PRODUCTION BY *CLOSTRIDIUM BOTULINUM* IN RAW REFRIGERATED FLOUNDER FILLETS

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Abstract

Prevention of toxin production by *Clostridium botulinum* is essential for processors of raw or unpastuerized vacuum (VAC) and/or modified atmosphere packaged (MAP) refrigerated fishery products. Packaging products under VAC and MAP conditions can significantly extend the shelf life of raw fish products. Since, there is commercial interest in marketing VAC and MAP refrigerated, (never frozen) raw fish fillets, the objective of this study was to determine if *C. botulinum* toxin development precedes microbiological spoilage in raw, refrigerated flounder fillets.

Aquacultured flounder (*Paralichthys dentatus*) fillets were either aerobically packed (Oxygen Transmission Rate (OTR) of 3,000 cc/m^2/24h@70°F), vacuum packed or MAP packaged in a 100% CO_2 atmosphere (OTR of 7.3 cc/m^2/24h@70°F). Fillets were stored at either 4 or 10°C. Spoilage was determined on separate fillets using microbiological criteria of bacterial counts >10^7 CFU/g (initial counts were 10^2 to 10^3 CFU/g). At 10°C, spoilage occurred at an average of 6, 8, and 15 days for aerobic, vacuum and 100% CO_2 packages, respectively. At 4°C, spoilage occurred at an average of 13, 30, and >37 days for aerobic, vacuum and 100% CO_2 packages, respectively.

Based on the time to spoilage, additional samples were analyzed qualitatively for botulinum toxin using a mouse bioassay. An external inoculation of approximately 10^2-10^3 non-heat shocked spores per gram of five strains of nonproteolytic *C. botulinum* (Type B 17B, Type E Beluga, Minnesota, Alaska and Type F 83) was used. This research will provide valuable information for predicting the microbiological safety of raw, refrigerated fish fillets packaged with modified atmospheres.

Introduction

Clostridium botulinum type E is a gram positive, anaerobic, spore-forming foodborne pathogen. It is a member of the non-proteolytic, psychrotrophic group comprised of *C. botulinum* B, F, and E. *C. botulinum* Type E is isolated from water, aquatic sediments and organisms. Estimated concentrations of *C. botulinum* Type E range from 1 - 200 per kg, although some studies have indicated 2,000-3,000 spores/kg (Lund and Peck, 2000). This organism is indigenous to the aquatic environment and fish is an excellent substrate for growth. Spores can survive cooking or adequate heating. The organism also has ability to grow at temperatures as low as 3.3°C (Eklund and others, 1982; 1992) or even down to 3.0°C (Graham and others, 1997). Eklund (1992) identified the following conditions for foodborne illness from *C. botulinum* to occur: the food must be contaminated with spores or vegetative cells; the processing treatment must be inadequate to inactivate spores or the product is recontaminated after processing; the food must support growth and toxin formation when temperatures exceed 3.3° C; and the food is consumed without cooking or after inadequate heating to inactivate preformed toxin.

The use of MAP and VAC can increase the shelf life of refrigerated fish products (Reddy and others, 1992). Shelf life is increased by the inhibition of aerobic spoilage bacteria, but VAC and MAP will not inhibit the growth of *Clostridium botulinum* (ICMSF, 1996). Fish inoculated with high numbers of spores and stored under VAC or MAP conditions have become toxic within 6-8 days during refrigerated storage when temperatures approached 50° F (10°C) (NACMCF, 1992). This is a concern, since in distribution and retail storage, product temperatures have been found to fluctuate between 40-50° F (4.4-10°C), (NACMCF, 1992).

Materials and Methods

Flounder (*Paralichthys dentatus*) fillets were derived from adult fully grown finfish located at the aquaculture facility of the Virginia Seafood Agricultural Research and Extension Center, Hampton, Virginia. All flounder were fed the same diets under the same growth conditions, and were the same age. Four boneless and skinless fillets were obtained from each fish (~450g). The fish were filleted, iced and immediately returned to the lab for packaging and analysis. Individual fish fillets were either aerobically packed (OTR of 3,000 cc/m2/24h@70°C which is considered a MAP by the FDA), vacuum packed or MAP in 100% CO_2 (OTR of 7.3 cc/m2/24h@70°C in the last two packages). Storage temperatures were 4 and 10°C. Microbiological analyses

included psychrotrophic plate count, aerobic plate count and anaerobic plate counts. Analytical procedures followed the Food and Drug Administration/*Bacteriological Analytical Manual*.

Individual inoculated fish fillets were packaged and stored as mentioned above. Sampling days for 4°C aerobic, vacuum and 100% CO_2 occurred on days 0, 20, 25, 30 and 35. Sampling days for 10°C aerobic occurred on days 0, 4, 6, 7 and 8. Sampling days for 10°C vacuum were on day 0, 7, 9, 10 and 11. Sampling at 10°C 100% CO_2 occurred on days 0, 12, 15, 17 and 18. Analyses included a qualitative examination for botulinum toxin. An external inoculation of approximately 10^2 -10^3 non-heat shocked spores per gram was applied by dropwise addition followed by spreading with sterile L-shaped glass rod into a thin layer (NACMCF, 1992).

Five strains of nonproteolytic *C. botulinum;* Type B 17B, Type E Beluga, Minnesota, Alaska and Type F 83 were cultured anaerobically and spore crops were created using procedures as described in Anellis, et. al.(1972). Spores were enumerated using serial dilutions in buffered peptone water and anaerobically incubated in TYGPT roll tubes then diluted to a final concentration of 10^2-10^3 /ml with sterile buffered peptone water then stored in vials at -80°C until needed.

Detection of *C. botulinum* toxin followed the general protocol as outlined in the Federal Drug Administration Bacteriological Analytical Manual (FDA, 1998b). Samples were pulverized in a laboratory stomacher for 2 minutes at 230 rpm with a 1:1 ratio of gel phosphate buffer (pH 6.2). Twenty milliliters of slurry were removed and centrifuged for 10 minutes at 10,000 rpm in a refrigerated centrifuge. Trypsin solution (0.2 ml) was added to 1.8 ml of supernatant for each tested sample (Trypsin was prepared with 0.5g Difco 1:250 trypsin being added to 10 ml sterile distilled water) The subsequent treated solution was incubated at 35-37°C with gentile agitation for 1 hour. Five 15-18g male mice (*Mus musculus)* (2 trypsinized, 2 non-trypsinized, 1 control) were given a intraperitoneal injection with 0.5 ml of supernatant. Mice were observed periodically for symptoms of botulism for 48 h. Botulism signs typically began within the first 24 h with ruffling of fur, followed in sequence by labored breathing, weakness of limbs, total paralysis with gasping for breath, and finally death due to respiratory failure.

Results and Discussion

Spoilage was determined on separate fillets using microbiological criteria for bacterial counts >10^7 CFU/g (initial counts were 100 to 1,000 CFU/g). At 10°C spoilage occurred at an average of 6, 8, and 15 days for aerobic, vacuum and 100% CO_2 packages, respectively. At 4°C spoilage occurred on the average of 13, 30, >37 days for aerobic, vacuum and 100% CO_2 packages, respectively.

At the 4°C incubation temperature, toxin formation did not occur after 35 days in any of the packaging combinations. The aerobically packed (OTR of 3,000cc/m^2/24h@70°C) fillets also failed to produce toxin even after spoilage at 8 days at 10°C. In the vacuum packages at 10°C, toxin production occurred after spoilage at day 11, but in the 100% CO_2 packages toxin formed before spoilage, between days 12 and 18.

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L 42. LEVELS OF TOXIC METALS IN CANNED SEAFOOD

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Abstract

The production of canned fish has decreased in recent years, however these products still present a large consumption in several European countries. It is well known that such products may contain some toxic metals such as mercury, cadmium and lead. In order to evaluate the levels of these three elements, canned tuna, frigate tuna, chub mackerel, sardine, eel, lamprey, squid, octopus, blue mussels and several seafood spreads were analysed (more than 1800 samples). The methodology used was flame atomic absorption (Cd, Pb) and cold vapour atomic absorption (Hg).

The mean of total mercury levels was 0.12±0.14 mg/kg wet weight, being the highest values obtained in canned tuna. However, these values did not exceed the proposed limit by EU for tuna (1.0 mg/kg). All analysed samples showed lead levels lower than the indicated limits. On the other hand, a few samples (less than 2%) of canned tuna and squid exceeded the limit values proposed for cadmium, respectively 0.1 and 1.0 mg/kg.

Taking into account the type of consumption of these products it can be concluded that canned seafood products do not represent a risk in terms of human diet.

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L 43. DETERMINATION OF QUALITY CHARACTERISTICS OF *HOLOTHURIA TUBULOSA* (GMELIN, 1788) IN TURKEY SEA DEPENDING ON PROCESS TECHNIQUE

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Abstract

There are around 500 species of sea cucumber but few (around 10-17) are valuable commercially. The common species are teatfish (*Holothuria nobilis*), sandfish (*Holothuria scabra*), blackfish (*Actinopyga miliaris*), lollyfish (*Holothuria atra*), deep-water redfish (*Actinopyga echinites*) and prickly redfish (*Thelenota ananas*). Sandfish and teatfish are the most valuable species due to their thick body walls.

In Turkey an Aegean region species, *Holothuria tubulosa*, is collected by divers and kept as alive in sea water until it will be processed. Methods used by Chinese people are applied in process technique of sea cucumbers. Processing steps consist of cleaning, gutting, first boiling, descumming, slitting, second boiling, sun drying, packaging and storage, respectively.

In this study quality characteristics of *Holothuria tubulosa* were tried to determine depending on process technique. In this respect, water activity (a_w) values and microbial quality of the sea cucumber were determined during sun drying period on different weather conditions (temperature, wind velocity, humidity). Nutritional composition (crude protein, crude fat, moisture, carbohydrate, ash) and chemical quality control analysis (total volatile base-nitrogen, mg N/100 g; trimethylamine-nitrogen, mg N/100 g; thiobarbituric acid, mg malonaldehyde/kg) were also determined before and after sun drying of *Holothuria tubulosa*.

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L 44. PATHOGENIC BACTERIA AND HYGIENE INDICATORS IN COD *(GADUS MORHUA)* PRODUCTS DESALTED AT LOW AND HIGH TEMPERATURES

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Abstract

There is still an old-fashioned and erroneous idea that pathogenic micro-organisms can not survive in heavily salted food products, and so good manufacturing practices, indispensable to other kind of food productions, are not necessary in the case of salted fish factories. Nevertheless, some pathogenic organisms can grow in salt concentrations higher than 10% NaCl, and many of them can survive in saturated saline solutions.

The objective of this work was to determine the occurrence of pathogenic flora and hygiene indicators in cod products desalted at low and high temperatures. Coliforms, *Escherichia coli*, enterococci, sulphite-reducing clostridia spores and *Staphylococcus aureus* were quantified in wet salted, dried salted and in dried salted cod after desalting at 4ºC and 20ºC. The presence of *Salmonella*, *Listeria monocytogenes*, *Vibrio parahaemolyticus* and *Pseudomonas aeruginosa* was also investigated. Higher levels of coliforms were observed in wet salted cod samples than in dried salted ones. Samples desalted at 20ºC showed higher contamination by these organisms, in opposite to samples desalted at 4ºC. The presence of *E. coli* was only detected in samples desalted at 20ºC. Enterococci were isolated from all kinds of samples, even from those desalted at 4ºC. Sulphite-reducing clostridia spores were detected only in dried salted cod samples and in samples desalted at 20ºC. *Staphylococcus aureus* was detected either from dried or wet salted cod, presenting levels as high as 10^2 cfu/g. Levels of approximately 10^6 cfu/g were observed in samples desalted at 20ºC. No sample showed contamination by *Salmonella*, *V. parahaemolyticus*, *L. monocytogenes* or *Ps. aeruginosa*.

Results reveal the need to implement good manufacturing practices in salted fish industry and the extreme importance of desalting under refrigeration conditions.

Materials and Methods

Approximately twenty pieces, with 10 mm width, were cut from both wet and dried salted cod samples weighting between 2-3 Kg and purchased from two local factories. One third of the dried salted cod pieces was aseptically desalted in standing water at 3±1ºC for 24 h and another was aseptically desalted in standing water at 20±1ºC for 24 h (fish/water, 1:6). A total of 64 samples were analysed according to the following scheme: 16 of wet salted cod; 16 of dried salted cod; 16 of dried salted cod desalted at 4ºC; and 16 of dried salted cod desalted at 20ºC. Six pieces were randomly selected for analysis and aseptically triturated. From this mixture, four 25 g aliquots were weight and taken. One aliquot was homogenised in 225 g of tryptone saline solution for 60 s and decimal dilutions were prepared for semi-quantification (ISO 7218 E, 1996) of coliforms, *E. coli*, enterococci, sulphite-reducing clostridia spores and *Staphylococcus aureus*. Coliforms were determined by inoculation of serial decimal dilutions in lactose broth incubated at 30ºC for 48 h. Positive tubes were subcultured to brilliant green bile broth and incubated at 30ºC for 48 h. *E. coli* was determined from coliform positive tubes, subcultured both for brilliant green bile broth and peptone water and incubated both at 44ºC for 48h. Enterococci were determined by inoculation of serial decimal dilutions in Rothe broth incubated at 37ºC for 48 h. Positive tubes were subcultured to Litsky broth, incubated at 37ºC for 48 h. Positive results were confirmed by streaking into KF streptococcus agar and tested for catalase. Sulphite-reducing clostridia spores were determined after treatment of serial decimal dilutions volumes for 10 min at 80ºC. Perfringens selective agar was poured into the tubes containing the inactivated inocula and incubated at 37ºC for 5 days. *Staphylococcus aureus* was determined by inoculation of serial decimal dilutions in Chapman broth, incubated at 37ºC for 48 h. Positive tubes streaked onto Baird-Parker agar, incubated for 37ºC during 48 h and confirmation was performed with coagulase test. The detection of *Salmonella*, *Listeria monocytogenes*, *Pseudomonas aeruginosa* and *Vibrio parahaemolyticus* was performed according to ISO methods. Whenever necessary, isolates were identified by API systems 20 E, 20 NE, 20 Strep and 20 Staph.

RESULTS AND DISCUSSION

Higher levels of coliforms were observed in wet salted cod samples than in dried salted ones. Lower levels of coliforms were detected in samples desalted at 4ºC, in opposite to samples desalted at 20ºC. The presence of *E. coli* was only detected in samples desalted at 20ºC, some of them with high levels (levels of approximately 10^3/g). Enterococci were isolated from all kinds of samples. Wet salted cod samples showed higher levels of these micro-organisms than dried salted samples. Desalting at 20ºC instead of 4ºC, favoured considerably the growth of these bacteria. Sulphite-reducing clostridia spores were detected only in dried salted cod samples and in samples desalted at 20ºC (Fig. 1).

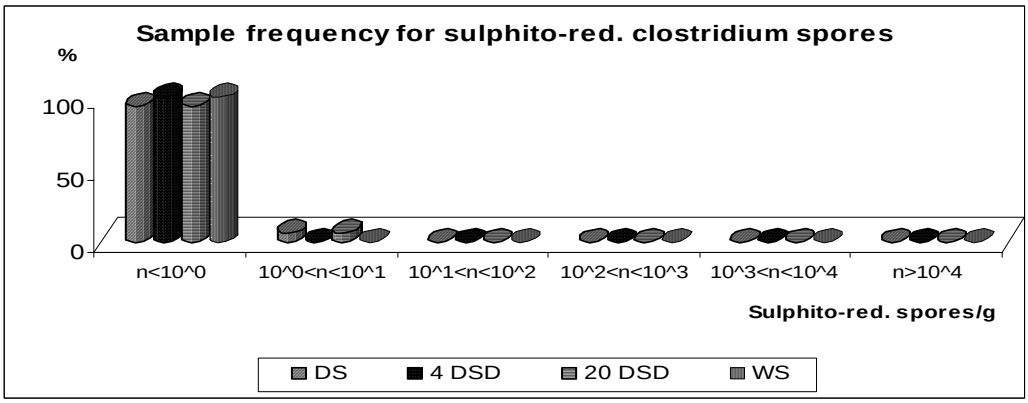

Figure 1 - Occurrence of sulphite-reducing clostridia spores in dried salted cod (DS), desalted dried salted cod at 4ºC (4DS) and 20ºC (20DS) and in wet salted cod (WS) samples.

Staphylococcus aureus was the main contaminant of dried and wet salted cod samples, presenting levels as high as 10 - 10^2 cfu/g (Fig. 2). Unsafe levels (<10^7 cfu/g) were observed in samples desalted at 20ºC, leading probably to toxin formation.

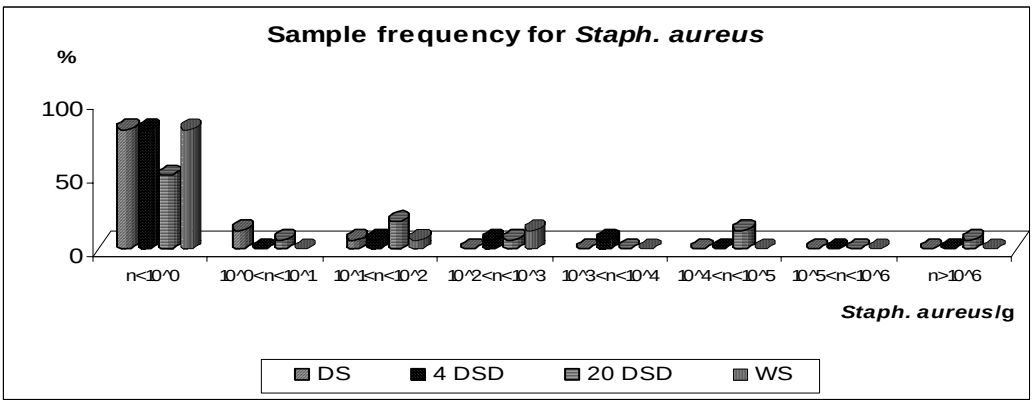

Figure 2 - Occurrence of *Staphylococcus aureus* in dried salted cod (DS), desalted dried salted cod at 4ºC (4DS) and 20ºC (20DS) and in wet salted cod (WS) samples.

No sample showed contamination by *Salmonella*, *Vibrio parahaemolyticus* or *Listeria monocytogenes*.

These results reveal the need to implement good manufacturing practices in salted fish industry and the extreme importance of desalting under refrigeration conditions. It is also essential to maintain low temperatures during storage of cooked products, as outbreaks due to *Cl. perfringens* have been related to consumption of cod specialties (Sciarrone *et al.*, 1997).

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L 45. ASSESSMENT OF BIOFILM FORMATION IN THE SEAFOOD INDUSTRY - INTERM RESULTS FROM A TWO YEAR PROJECT

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Abstract

The aim of this project is to improve the microbial safety of seafood by improved design and cleaning instructions of processing lines and machinery. The project is based on evaluation of hygienic design in the industry as well as on research on biofilm, which will be investigated in the natural working environment on the one hand and in a laboratory on the other. Results presented here include the first part of this project where microflora of biofilm in seafood processing line was characterized as well as biofilm formation of mixed culture of *Listeria monocytogenes* with some strains isolated from the seafood processing line was tested on different treated stainless steel surfaces. No significant differences were observed between different treated steel surfaces related to the roughness but in all cases increased bacterial adhesion occurred with increased contact time. In the presence of *Pseudomonas* spp. colonisation of *L. monocytogenes* on stainless steel was significantly enhanced.

Introduction

Attachment of pathogens and other bacteria to food equipment surfaces can lead to product contamination, spoilage, and surface destruction. Research in the food industry has indicated that in natural habitats most bacteria are able to colonize surfaces. Bacteria are natural part of the raw material for food production but they can also establish in the food processing equipment where they can contaminate the products (Fonnesbech Vogel et al. 2001). In ecosystems the pathogens work together with other bacteria. Because of this relationship then traditional cleaning and disinfecting procedures do not always give satisfactory results. Attached bacteria are reported to have enhanced resistance to disinfectants compared to free-living cells (Bourion and Cerf, 1996). Some Gram-negative bacteria *(e.g. Pseudomonas* spp.) have some resistance mechanisms against some disinfectants (e.g. QUATS). The choice of specific materials for the processing equipment can also influence if and how much microorganisms accumulate in critical places, which can lead to the forming of the so-called biofilm. *L. monocytogenes* is a foodborne pathogen of major public health concern. It is very difficult to control this organism in the food processing environment. This pathogen can adhere to food contact surfaces in the processing environment, but it is not likely that *L. monocytogenes* will be present as a single-species culture. This is important, as the presence of other bacteria, e.g. *Pseudomonas* spp. has been reported to enhance number of *L. monocytogenes* on a surface and the resistance of them to disinfectants (Bourion and Cerf, 1996). The greatest challenge in controlling *L. monocytogenes* in the food industry is therefore to prevent establishment of the bacterium in specific niches, where routine cleaning and disinfection are ineffective.

Materials and Methods

Processing environment - Stainless steel samples where placed near to food contact surfaces in different location in a shrimp factory (16 samples) and a fish factory (10 samples). Both plants use similar cleaning procedure involving water rinsing, followed by caustic wash in 0.5% NaOH and water rinsing before disinfecting with quaternary compounds. An acid wash was conducted weekly in the shrimp factory. After 3 months in the factory the samples where removed and transported to a laboratory and biofilm sample taken from each surfaces with a cotton swab. The cotton swab was blended with 10 ml of Butterfield´s buffer. Serial dilutions were prepared and sample plated on Iron Agar (Gram et al., 1987). The plates were incubated for 15°C for 7 days. Twenty five colonies were isolated at random and identified by different test to genus level (Gram stain, form and size, catalase, oxidase, motility, oxidation /fermentation of glucose).

Laboratory work - Bacterial adhesion of mixed culture (*L. monocytogenes* and a Gram negative rod) to different treated stainless steel coupons where tested and compared. Coupons were soaked in 1N NaOH overnight to etch the surfaces and after that for one hour in acetone to remove any grease. Each coupon was placed vertically in a glass tube. The steel coupons were finally autoclaved at 121°C for 15 min. Into an autoclaved glass tube 30 ml of sterile shrimp juice was transferred. Sterile shrimp juice is more close to the conditions in the seafood industry compared to nutrient broth. One ml of relevant bacterial suspension (10^3-10^4 cfu/ml) was pipetted to the shrimp juice. Contact time was 24, 72 and 120 h. Incubation was at 18-21°C with shaking at 70 rpm. Tests were performed in triplicate. After contact time, coupons were removed aseptically form test suspension and rinsed gently with 3 x 50 ml of sterile water to remove unattached cells. Two different methods were used to remove the bacteria, swabbing method and ultrasonication (Bourion and Cerf, 1996). Serial dilutions were prepared and sample plated on Tryptic Soy Agar with 0.6%Yeast Extract (Difco) for total count and Modified Oxford agar (MOX from Difco) for *Listeria* count.

Results and Discussion

The microflora in biofilms formed under real conditions on stainless steel surface which were planted in different location in a shrimp factory (16 samples) and a fish factory (10 samples) was identified. A total of 391 and 175 isolates, respectively, was obtained from shrimp and fish factory, both showing a higher proportion of Gram-negative rods (81 and 71%). The results show that biofilm were found in a variety of location and the population densities remaining after cleaning was 10^2-10^5 cfu/cm^2 on samples taken from shrimp factory and 10^5-10^6 cfu/cm^2 on samples taken from fish factory. These results show that regular cleaning and disinfection limit the progression of attached cells to extensive biofilm on food contact surfaces especially in the shrimp factory. The large biofilm structures common in other industries (multilayer of bacteria, typically above 10^8 cfu/cm^2), rarely occur in food processing environments (Holah and Gibson, 1999). The predominant genus attached to the surfaces was *Pseudomonas* spp. (60%) in the shrimp factory and *Enterobacteriaceae* (22%) in the fish factory. The other Gram-negative flora included *Aeromonas, Moraxella* and *Acinetobacter*. The Gram-positive flora from both plants included *Micrococcus* spp. and coryneforms. The presence of *Listeria* spp. was determined with negative results in all cases. Three different Gram-negative strains where selected to study attachment of *L. monocytogenes* in mixed culture on three different treated stainless steel surfaces. Highest number of attached bacteria to different treated stainless steel surfaces where obtained after contamination with mixed culture of *Serratia liquefaciences* and *L. monocytogenes*. A lower number of bacteria adhered on the steel surfaces when mixed culture of *Pseudomonas* spp. and *L. monocytogenes* and mixed culture with *Aeromonas* spp. and *L. monocytogenes* were tested. On the other hand in the presence of *Pseudomonas* spp. colonisation of *L. monocytogenes* on stainless steel was significantly enhanced and that is consistent with previous data (Bourion, 1996).

After 96-120h no significant difference was observed between different surfaces related to the roughness but the time factor influenced greatly with more adhered bacteria related to prolonged contact time.

Conclusions

Micro-organisms can attach and grow relatively rapidly on different kind of stainless steel surfaces in the food processing environment, although this development is generally limited by cleaning and disinfection procedure. Numbers of adhering bacteria recovered from the surfaces indicate that in these cases a monolayer of bacteria adhere to the surface but not a multilayer which is known in other industry. Regular cleaning and disinfection limit the progression of attached cells to extensive biofilm on food contact surfaces especially in the shrimp factory.

Micro-organisms attached to surfaces may be an important source of contamination and it is therefore important to improve the design of processing equipment with hygienic requirement in mind. The choice of specific materials and their surface treatment are important factors to have in mind when food processing equipment is designed but other hygienic design criteria like welding, joints, dead ends and sharp corners can be of more importance.

Acknowledgement

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L 46. EPIDEMIOLOGY OF *LISTERIA MONOCYTOGENES* ALONG A RAINBOW TROUT FOOD CHAIN PRODUCTION

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Abstract

The aim of this study was to identify the origin and to characterise *Listeria monocytogenes* strains isolated from different sites located along a river.

Three sites 'A, b and C) were investigated during winter 2002. One hundred and two samples were collected by swabbing different places of the fish farms (A and B) and materials in the slaughter plant C, and by sampling live (mucus, fillets, gills…) and eviscerated fishes. One litre of water from upper and down river was also samples at each site.

All samples were analysed for *Listeria monocytogenes* detection using an enrichment (Fraser broth) and isolation (ALOA medium) procedures, then typical colonies were identified using Microgen-Listeria-ID test. *Listeria monocytogenes* strains were characterised by Pulsed Field Gel Electrophoresis (PFGE) technique using *Apa1* and *Asc1* enzymes.

Thirty-eight samples were contaminated by *Listeria monocytogenes*. Concerning farms A and B, *Listeria monocytogenes* was present not only in the water upstream but also in the environment and mainly on the surface (mucus) of live fishes. In the slaughter plant, *Listeria monocytogenes* was found in the environment, especially during the first investigation, and again, on the surface of fishes.

Results concerning the restriction enzymes profiles confirm the diversity of strains isolated along the river and the difference between these variable clones and one specific present only in the processing plant, colonising the equipment.

Introduction

Listeriosis is a very serious foodborne disease affecting preferentially pregnant women, new-born infants and immunocompromised patients (Rocourt *et al.*, 2000). Therefore, fishes and fish products were sometimes associated with outbreaks or sporadic cases of human listeriosis and, specially in two cases in relation with vacuum-packed gravad and cold-smoked rainbow trout preparations (Ericsson *et al.*, 1997, Miettinen *et al.*, 1999, Tham *et al.*, 2000).

Listeria monocytogenes (*L. monocytogenes)* occurs widely in different environments including fish production farms and slaughter plants. In that way, *L. monocytogenes* can contaminate the processing line and finished products (Johansson *et al.*, 1999). Due to the diversity of origin along the food chain, it is important to trace the different source of contamination for a better understanding of the epidemiology of these different strains. For that, the use of DNA-based techniques and specially Pulsed Field Gel Electrophoresis (PFGE) have proved to be a very interesting method to discriminate between strains of *L. monocytogenes* (Johansson *et al.*, 1999.

The aim of this study was to examine the occurrence of *L. monocytogenes* in different sites of rainbow trout production situated along a river, and to characterise isolated strains by PFGE in order to determine the origin and the epidemiological diversity along the river.

Materials and Methods

Three sites (A, B and C) were investigated during winter 2002. One hundred and two samples were collected by swabbing different parts of the 2 fishes farms (A and B) and equipment in the processing plant (C), and by sampling live (mucus, fillets, gills…) and eviscerated fishes. One litre of water from upper and down river were also sampled at sites A and B. In total, 7 series of sampling were realised as follow: one on site A and 3 on sites B and C. For the latest sites (B and C), series of sampling were made on the same day (2 and 5; 3 and 6; 4 and 7). All samples were analysed for *L. monocytogenes* detection using an enrichment (Fraser broth) and isolation (ALOA medium) procedures, then typical colonies were identified using Microgen-Listeria-ID test. *L. monocytogenes* strains were characterised by Pulsed Field Gel Electrophoresis (PFGE) technique using *Apa1* and *Asc1* enzymes.

Results and Discussion

L. monocytogenes was isolated from 38 out of 102 samples (37,25 %) (Table 1). A high number of positive samples (14/30) were found at the farm level situated up the river (site A). In this farm, *L. monocytogenes* was present on the surface (mucus) of the 4 fishes analysed and on different places around the farm such as walls of tanks, nets used to catch fishes… More important, *L. monocytogenes* was also present in the water (1 litre)

sampled in the river, just before entering the first basin of the fish farm. Nevertheless the other 3 water samples issued from other places, including the downstream side of the farm were negative. In addition, the water contamination, only one sample corresponding to water entering the farm, was positive in the second site.
In the slaughtering plant, during the first series, *L. monocytogenes* was present (9/12) in the environment and especially on different conveyor bells and boxes. Nevertheless, the occurrence was lower during the second (3/18) and the third (2/21) series, due to the improvement of the cleaning and disinfecting procedures and of the hygienic conditions during slaughtering.

Table 1: Results of *L. monocytogenes* from different sites and samples.

Sites	A		B			C	
Series	1	2	3	4	5	6	7
Fishes *	8/17 (4/4)	1/4 (1/1)	1/4 (1/1)	0/4 (0/1)	2/7 (1/2)	2/3 (1/1)	0/10 (0/3)
Environment	5/9	2/2	1/1	1/2	9/12	1/15	2/11
Water	1/4	1/2	0/1	1/4	-	-	-
Total	14/30	4/8	2/6	2/10	11/19	3/18	2/21

* ( ) : Number of positive fishes out of number of analysed.

Concerning the mode of sampling, it seems that applying swabs (towels drunk with the enrichment broth) on the surface of fishes or on materials, is a very interesting methodology. In that way the mucus sampled on the surface of the fish is very useful (Eklund *et al*., 1995).

Combination of *Apa 1* and *Asc 1* results allows classifying isolates into PFGE types. Seven types were identified among 44 strains tested (table 2). Two types are predominant: the type 18 seems to be a common type, but was present only in the processing plant (table 3). The second one (type15) corresponds to the serotype 4b. These strains were present in the 2 farms, not only on the surface and intestines of fishes, but also in the environment, i.e. the net, and in the water. The other types are less common but one of them (19) isolates once on the surface of one fish belongs to the "yellow clone" known to be associated with foodborne outbreaks and sporadic cases (Tham *et al*., 2000). In general, it seems that upstream the river, the number of PFGE types (4) is higher than downstream.

These results confirm the ubiquitous diversity of clones of *L. monocytogenes* present in the environment. Nevertheless there is a difference between genotypes isolated from the fish farm and from the processing plant. At this place, only one type was present (type 18) confirming that strains present during the primary production do not get over the other during the processing step (Dauphin *et al*., 2001). It is difficult to explain these results because, in this study, the farm B and the processing plant are adjacent. The presence of the same type (18) in the processing plant after several weeks and whether the improvement of hygienic procedures, confirms the ability of different resident strains to survive and colonise surfaces of equipment. In that way, comparing these strains with the others present in the fish farm could be very fruitful.

Table 2: Number of isolates of *L. monocytogenes* according to PFGE types.

PFGE Types	N° of isolates
11	1
15	10
17	5
18	22
19	1
30	2
32	3

Table 3: PFGE types of *L. monocytogenes* isolated from different sites.

Sites	A	B		C	
Series	1	2	3	4	5
Fishes	11-15-30-32	15	15-19	18	18
Environment	15-17-30-32	15-17	17	18	18
Water	30	15	-	-	-

Conclusions

In this study concerning one epidemiological unit including 2 farms and 1 processing plant situated along a river, the occurrence of *L. monocytogenes* varied considerably. In the 2 fish farms, *L. monocytogenes* was present not only on the surface of fishes but also on the equipment and in the water. Concerning the processing plant, the occurrence of *L. monocytogenes* decreased when improving the cleaning and disinfecting procedures and the hygienic conditions. The characterisation of strains of *L. monocytogenes* by PGGE confirms the diversity of these bacteria, especially in the fish farms. Nevertheless, in the processing plant, only one type, different from the others, was present, colonising the equipment during several weeks, despite the improvement of hygienic conditions. This fact could be explained by differences between these clones for colonising or surviving in different environments.

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L 47. DEVELOPMENT OF TEMPERATURE LOGGING TECHNOLOGY FOR THE FISHING INDUSTRY

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Abstract

Maintaining the quality of chilled fish at sea is very important for maximising shelf life and value. One of the major tools at our disposal for ensuring quality is remote temperature monitoring. Today, fishermen are legally obliged by EU Directives and Government Legislation to implement temperature monitoring systems on-board fishing trawlers. Coupling temperature sensors with new technologies developed by the communications industry opens the door to temperature sensing systems that are autonomous, low power, and capable of transmitting data to, and receiving instructions from, remote base stations. Autonomous sensing is necessary in order to realise the full potential of temperature profiling at sea as well as maximising quality control and traceability. We have developed a unique temperature monitoring system that incorporates both Radio Frequency (RF) and Global Systems for Mobile (GSM) or satellite communications with temperature logging units, enabling temperature data to be easily accessed onshore while the trawler is still at sea. The temperature data is transmitted to a base station on board the fishing trawler via RF communications. The base station is connected to a GSM modem phone that allows the temperature data to be continuously transferred to an on-line website. Parameters such as time-temperature integration and transmission of data to shore facilitate quality control and traceability and could prove to be the core of quality assurance systems for the future. The availability of online time-temperature profiles will enable accurate estimations of shelf life to be made as the fish pass through the distribution chain, reducing the margins of error and thereby maximising the value for producers, while simultaneously improving consumer confidence in the product.

Introduction

Recent technological advances have made the dream of inexpensive, powerful, ubiquitous sensing a readily achievable reality. The convergence of sensor technologies, communications and computing has the potential to overcome barriers of time, scale, materials and environment (Girod and others 2002). This ubiquitous sensing capability carries some potentially revolutionary consequences for all types of businesses. Imagine if it was possible to track every item as it moves along the value chain from manufacturing all the way to the consumer. While these ideas may sound somewhat far-fetched, the technology exists today to implement this concept. Sensors have a pivotal role to play in this world, as they are the primary sources of information for the networked world. Autonomous sensors are currently being developed that integrate wireless communications within miniaturised sensing devices. The revolutions in wireless communications and sensors bring many opportunities in remote sensing (Estrin and others 2001). One area of great importance is the evaluation and monitoring of the quality of food we eat. This summary report explains how newly developed technology can bring data collection and traceability to the next level where a future vision sees extensive sensor networks working together to revolutionise traceability and quality control in the food industry.

F/GSM Temperature Monitoring System

An autonomous temperature monitoring system has been developed to transmit detailed temperature data of fish catches ashore using RF and GSM communications. GSM phones provide an accessible means of transmitting data from smaller inshore vessels that are relatively close to land, compared to more expensive satellite communications used in deep-sea vessels. However, the GSM system is perfectly adequate for demonstrating the feasibility of our system, and it can be easily transferred to a satellite system when it has been refined. A schematic of the temperature monitoring system can be seen in figure 1.

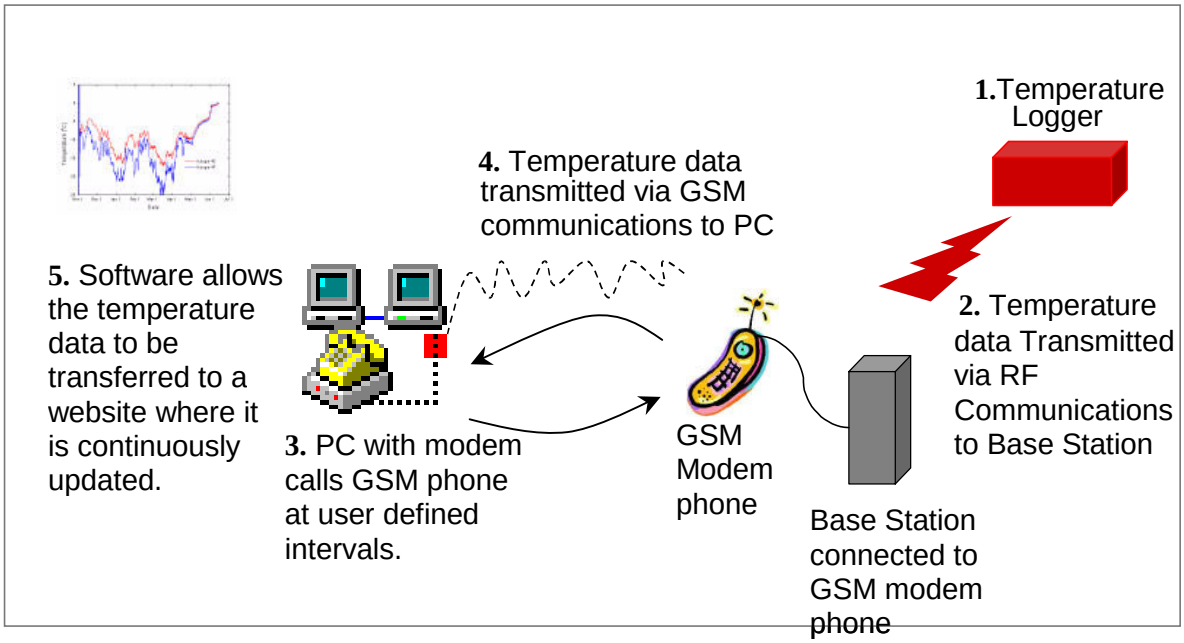

Figure 1 Schematic of RF/GSM Temperature Monitoring System

The temperature logger used has an accuracy of 0.1ºC, is battery powered and has programmable parameters such as start time and measurement frequency. The logger has adequate data storage capabilities and the temperature data is date and time stamped. It is completely sealed and robust to withstand the harsh sampling conditions at sea. The logger, completely submerged in a container of fish, records the temperature of the bulk of the fish in real time (step 1) from point of catch to point of sale in auction. While still at sea the logger communicates the temperature data to the base station on board the fishing trawler via RF communications (step 2), which has an operating range of 10 metres. The base station is connected to a GSM modem phone that allows the temperature data to be transmitted to a shore-based computer (steps 3 and 4). Here, the data is automatically processed using specially designed software that uploads the temperature data onto a password protected web page (step 5, see also figure 2). This system allows the temperature data of the fish catch to be accessed from anywhere in the world while the fishing trawler is still at sea.

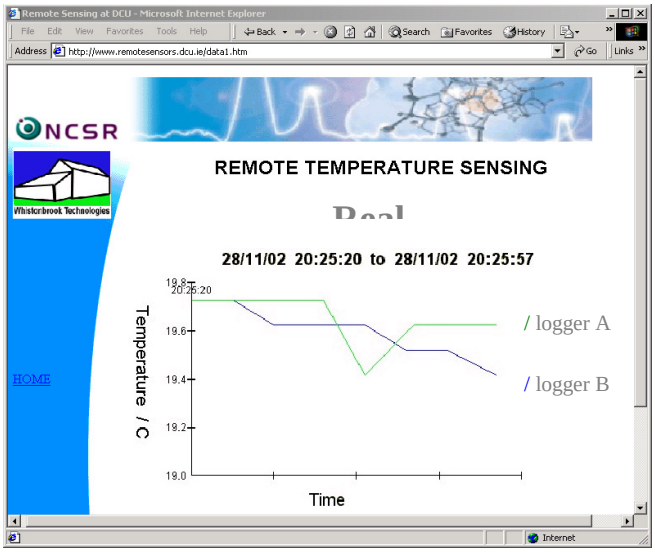

Figure 2. This web site displays the real-time temperature data obtained by the RF/GSM Temperature System from a remote location.

Conclusion

Today, as electronic auctions are becoming more and more popular it is important that quality is monitored and assessed on board the fishing trawlers (Crick Carleton 2000). With the electronic auction system, fish lots have to be accurately described so that the remote buyers know exactly what they are buying and do not suffer any disadvantage relative to someone buying on sight. This information can be provided by the proposed autonomous, wireless sensing system, which gives a detailed and accurate time-temperature history of the catch. Once the necessary procedures for quality control have been implemented on fishing trawlers, quality control and product traceability will become readily available. In the future, such a RF/GSM Temperature Monitoring System will prove invaluable to the fishing industry.

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L 48. THE IMPLEMENTATION OF CLEANER TECHNOLOGY IN FISH CANNING INDUSTRY

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Abstract

Proceedings leading to the introduction of cleaner technology in one of the biggest in Poland canned fish producers factory have been presented. The methods utilized allowed to decrease the sewage indicators by approx. 80% (i.e.: BOD_5 decreased from 18400 mg/l to 2200 mg/l). In the process of purifying of post production water in 2002, 270 tons of fish oil has been regained, which proved to be a very good component for swine and chicken fodder. Additionally during the same purification process low fat and condensed protein coagulate is being obtained, where in 2002 the factory regained 1800 tons. The following research has shown that the coagulate may be used as fodder component or after composting as a mineral – organic feed. The utilization of fish waste products after preliminary processing allowed to gain approx. 1300 tons of protein high fish meal annually, and the condensing and centrifuging of stick water allows to gain additional fish oil making the production process waste free.

Introduction

A significant obstacle for the development of industry including fish processing industry may be the requirement to fulfill the environment protection requirements. One of the solutions for that problem is the introduction of the so called cleaner technology (CT), and specifically the basic CT tool which are the WMA procedures (Waste Minimization Assessment). This is an integrated method for redesigning and reorganizing of a production facility so it processes products without or with low waste ratio (gathering and securing waste products where those are created).

Environment protection in fish processing industry has never had a high priority and the solution to that problem requires extensive investments, which in many cases exceed the producers financial capabilities. One of the solutions to resolve that problem may be to utilize the vitamins and proteins rich waste products and post production water at the same time purifying the sewage with low waste product ratio.

The Polish fish processing industry consists of 350 production facilities varying in sizes, which produce approx. 12 million m^3 of sewage. Only several factories are equipped in modern mechanical – chemical sewage treatment plants. Some factories are equipped only in mechanical sewage treatment facilities, but mostly – especially smaller factories gather the sewage in sedimentation containers and then delivers those to municipal waste treatment facilities.

The producers of fish products have in large part managed to deal with the fish waste products problem, utilizing those for the production of hydrolyzed proteins or fish meal or in some cases direct utilization in feeding of farm fur animals. A serious environmental problem is the sludge created in the process of waste products treatment. The character of the sludge depends on the treatment procedure. Those could be blasties from grids, rotosieve waste, sludge after floatation, activated sludge or old fat from installations. All those waste products after appropriate treatment could be utilized for fodder, feed or technical purposes. The direction in which those products are utilized depends on their chemical composition and the conditions in which the products were obtained [1].

In one of the biggest Polish canned fish production factories (approx. 10000 tons annually) a mechanical – chemical post production water treatment plants has been put to service, where the problem of both the treatment and utilization of sludge obtained in the treatment process has been resolved. In order to utilize the fish waste products and the created in the treatment process protein – fat sludge, a fish meal production facility has been constructed. The introduced steps allowed to obtain very satisfying effects as to the reduction of basic contamination indicators in the factory sewage as well as for the creation of conditions allowing for the direct utilization of sludge after floatation [2].

Method and Materials

The introduction of cleaner technology elements in the canned fish production factory was based on:

- The utilization of fish offals in the on site fish meal production facility and a direct utilization of stick water,
- Utilization of mechanical – chemical method of post production water treatment,
- The utilization in the fish cans production line of a blancher with a construction allowing for the collection of the thermal drip.

The post production water treatment was based on:

- Averaging the composition of equalizing tank contents,
- Coagulation using iron or aluminum salts,
- Floatation supported by pressure saturation of the created protein-fat coagulate (coagulate dry mass approx. 21%),
- Compacting of the coagulate in a filtration press to 36% of dry mass.

As a result of the process we obtain:

- Raw fish oil,
- Protein coagulate,
- Sewage where the fat and proteins have been separated from, which is then directed to the regional sewage cleaning station.

From the raw fish oil after heating to 70^0C and centrifuging we obtain feed fish oil (FFO). The protein coagulates can be mixed with grain or grass or with fish offals and directed to the fish meal production facility where we can obtain modified fish meal. The protein coagulates can also be used in the production of bio-compost.

The analysis of the basic composition of the obtained products was conducted according to Research Procedures for MIR Certified Research Laboratory. The chemical characteristics of sewage was conducted according to Polish norms.

The fatty acids were determined using the classic gas chromatography method: a Philips chromatograph fitted with a flame-ionization detector. An Rtx – 2330 column 105 m in length was used. The temperature in the spray chamber was 220^0C, and in the detector it was 230^0C. Helium (70 psi) was used as the carrier gas. These tests were conducted in the analytical laboratories of the Agricultural University in Wroclaw.

The contents of minerals and trace elements, including heavy metals, were determined by the ICP method with plasma spectrometer with an ICP-MS Varian Ultra Mass – 700 mass detector. The mercury content was determined with a AMA-254 spectrometer, and an Orion Research EA-940 ionometric gauge was used to determine fluorine content. Samples were mineralized using the microwave technique with an MDS-2001 (CEM) station according to the standard procedures of the Environmental Chemistry Laboratory (ICP Certificate no. 01.04.15) for the Agricultural University in Wroclaw.

The levels of fat soluble vitamins (A, D_3 and E) were determined by particle chromatography with a device from the Merck-Hitachi company which was equipped with a fluorescence detector and a DAD (Diode Array detector). This is the method applied in the Accredited research laboratory of the Sea Fisheries Institute in Gdynia (accreditation certificate no. L 17.4.990 confirmed by the Polish Research and Certificates Center) – methodology instruction no. IM-24.

The levels of organochlorine pesticides (OCPs) and polychlorinated biphenyls (PCBs) were determined using a method which was developed at the accredited Research Laboratory at the SFI based on specialist scientific literature (research procedure no. PB-10). This method is based on extracting the compounds with hexane and then cleaning the extracts with sulfuric acid and 30% oleum. Capillary gas chromatography with an electron capture detection was used to analyze the compounds quantitatively (apparatus by Fissons).

Conclusion and Discussion

1. Reduction of pollution indicators in sewage after cleaning.

The implemented in the factory solutions (chemical – mechanical treatment, mechanical preliminary processing, direct utilization of stick water from the fish meal factory) allowed to greatly reduce the contamination indicators in the sewage from the factory. The reduction in the basic indicators levels, based on a daily research, has been presented in the following table.

Indicators [mg/l]	Sewage	Sewage after cleaning	Reduction [%]
BZT_5	18400	2200	88
CHZT	29200	3400	88
Suspended matter	3600	590	84
Ether extract	9300	80	99
Nitrogen total	930	390	58

The research conducted in the laboratory allowed to obtain such decrease in basic indicators (BZT_5 under 2500 mg/l) that the factory received an official allowance to transport sewage after cleaning to the local sewage cleaning station.

2. Feed fish oil (FFO)

The results of physical and chemical research of raw FFO show that all researched parameters such as color, water content, undiluted substances in ether (SNEN) and nitrogen(proteins) were low, and did not exceed the maximal values. The most important parameters such as AV and PV, which point at the hydrolytic oxidative and polymeric transformations also did not exceed the values stated in the norms.

The composition of the oil is quite interesting. It has been stated that FFO has a high content of unsaturated fatty acids (UFA) – 82.72%, polyunsaturated fatty acids (PUFA) – 35.59% including the omega-3 group, with a favorable omega-6 omega-3 ratio which reached 1.07. Considering the animal feeding patterns the composition of fatty acids is quite good mainly due to high content of PUFA and omega-3.

The content of liphophylic vitamins in FFA is quite high (vitamin A – 458-IU/g, vitamin D_3 – 240 IU/g of oil, vitamin E – 1.21 mg/g of oil). As a comparison in 1 ml of oil (from cod liver) there is 920 IU/g of vitamin A, 92 IU/g vitamin D_3 and 0.28 mg vitamin E. Because of the usefulness of those vitamins for pigs, broilers and ruminants there is a possibility to mostly cover their needs in vitamin D_3, in less extent vitamin A using even small FFO quantities.

The FFO has been analyzed many times determining its chemical composition in the direction of organochlorine pesticides (COP) and polychlorinated biphenyls (PCB), which is easily stored by animal cells creating a serious threat even to the human health. The DDT sum did not however exceed the allowed in EU norms as stated in EU Commission Directive no. 1999/29/EC, The PCB content (8 congeners) also did not create toxicological threats. The total amount of those did not exceed the EU allowed values stated in EU Commission Directive no. 99/449/EEC.

The micro-biological research clearly show that FFO is not a good feed for microorganisms. The research in the direction of *Salmonella* and *Clostridium Perfringens* was negative, and the general number of moulds in fresh FFO and in FFO after 30 days storage did not exceed the Polish standards.

Based on the conducted research in the fields of chemistry, microbiology and toxicology it can be stated that the feed fish oil can become a good energy, fat and liphophylic vitamins source, especially for ruminants [3].

The feeding usefulness of fish oil has been determined based on biological tests conducted on farm animals (the research encircled 215 pigs and 180 chicken broilers).

According to the research into the pigs and broilers development it has been stated that fish oil additives to the feed compounds has shown positive production results and the increase in the farm effectiveness [4].

The results of research in the fields of chemistry, toxicology, microbiology, veterinary hygiene and feeding were the basis for the development of registration documentation of FFO. This documentation has been evaluated by three independent scientists in the field of veterinary sciences and animal feeding, who gave their positive opinions as to the possibility of using FFO for general use in feeding of farm animals (pigs and broilers)

3. Modified fish meal (MFM)

The research conducted under the supervision of Professor Z. Dobrzanski from the Agriculture Academy in Wroclaw has shown that MFM is a good source of proteins. Among the exogenous amino-acids it has large quantities of lisine (14.37%) and threonine (7.82%) as well as cystine, methionine and trypthophan (below 5%). The proteins from fish raw material is characterized by high biological value, especially the high true digestibility coefficient of vital amino-acids. So MFM can be used for the balancing of proteins in feeding of mono-gastric animals (pigs, broilers), even though its content is lower in comparison to other animal feeds.

The fatty acids content in MFM fat is an interesting question. The saturated fatty acids are only 36.03% and the unsaturated 63.97%. The content of linolenic acid is high (11.24%) as well as the omega-3 fatty acids, which in view of feeding physiology are quite useful, which is proven by many authors researching different feed and raw fish materials in the feeding process of pigs and broilers.

Vitamins are also important, where the MFM content is: vitamin A- 243 IU/g, vitamin D_3 – 38 IU/g, vitamin E – 0.29 mg in 1 g of extracted fat.

The research of macro elements content and trace elements content has shown that MFM is a good source of phosphor – 1.6%, magnesium – 0.16%, calcium – 0.84% and chloride and sodium . Among the microelements the attention is directed towards Zn (approx. 850 mg/kg), Fe (approx. 650 mg/kg), Mn (approx. 200 mg/kg) as well as the presence of Co, Cr, Cu, Mo and Se. Most of those elements are subject to norms in monogastric animals feeding because those fulfill important physiological tasks. The relatively high level of copper in MFM (approx. 50 mg/kg) and zinc (approx. 850 mg/kg) are not a toxicological concern because pigs and broilers tolerate those elements in feed, copper up to 250 mg/kg and zinc 1000 mg/kg. The toxic elements content (As, Hg, Cd, Pb, F) did not exceed the limits set in the Directive no. 1999/29/EC from 22.04.1999.

The research of organochlorine pesticides (OCP) content show clearly that in MFM those are not a toxicological threat. I.e.: the ΣDDT content (0.27 mg/kg) does not exceed the limits set forth in the Directive 1999/29.EC of 22.04.1999 (for feed and feed materials). Similarly, the PCB levels were also not very high, the ΣPCB_8 value was on average 0.122 mg/kg, which is allowed by the EU (Commission Decision no. 99/449/EEC of 09.07.1999).

MFM is a safe product also in the aspect of microbiology. The presence of *Salmonella* and *Clostridium* has not been determined, and the average content of moulds after 30 days of storing in normal conditions was 40000/g. According to Polish Standard the general allowable content of moulds in feed and feed raw products should not exceed 200000/g.

Summarizing the chemical, toxicological and microbiological research it can be stated that MFM is a valuable feeding ingredient and can become in the feeding of pigs and broilers a valuable source of fat and proteins as well as vitamins from the A group and D_3 also several macro elements (Ca, P, Mg) and Microelements (Zn, Fe, Mn, Cu, Se) [5].

The feeding usefulness of MFM has been determined based on biological tests on farm animals (162 pigs and 180 broilers).

Based on the conducted research it has been stated that the addition of MFM to feed mix had a positive impact on production and stock effectiveness. [6,7]

The results of chemical, toxicological and microbiological as well as veterinary hygiene research and nutritional research has been the basis for the creation of registration documentation for MFM. This documentation has been evaluated by three independent scientists in the field of veterinary sciences and animal feeding, who gave their positive opinions as to the possibility of using FFO for general use in feeding of farm animals (pigs and broilers)

4. Bio-compost

The compost has been obtained from concentrated post floatation sludge created in the process of coagulation post production water. As a result of the conducted tests and research into literature a compost has been produced which than was evaluated. In order to maintain the appropriate coal – nitrogen ratio in the manufactured compost (C:N=30), the mass for composting has been mixed (using layer mixing) together with fine cut grass and peat in the proportion of 6:1:1. the compost mass (because of low bio degradation of fat) strains of bacteria for decomposition support have been added. The available on the Polish market bacteria strains proved that the best solution was "Trigger" in quantities of 2 kg per ton of mass for composting. During the composting process the pH value has been modified using KOH. The process has been concluded after 80 days where the mass decreased significantly, decrease in fat and organic matter in comparison to dry mass and change of the composition from wet to dry [8].

The compost obtained in that process later used as fertilizer in experiments contained:

- Water 14%
- Whole nitrogen 2.18%
- Ammonium nitrogen 1.35%
- P_2O_5 4.66%
- K_2O 3.40%
- Ca 12.12%
- Mg 0.41%
- Na 0.21%

Along with the basic macro elements the compost contained also microelements such as: barium, copper, zinc, manganese, iron and molybdenum. Furthermore, the compost obtained in the process fulfills all Polish criteria as to the heavy metals content and later use for fertilizers.

The obtained fertilizer from fish waste products had correct fertilizing effect and has proven to be useful as additional (30-40%) fertilizer to cover the needs of plants. This fertilizer was especially good in the aspect of phosphor content and the quantity of fertilizer should be adjusted to the requirements of the plants and soil for phosphor. The quantity of 1.5 tons of compost per 1 hectare should not be exceeded.

The rye harvest in the field with compost was in accordance with compost quality from 9 to 13% higher than that in the control field [9].

Conclusions

The introduction of cleaner technology in the canned fish production factory allowed to:

- Greatly reduce the sewage pollution indicators in the sewage after cleaning,
- Utilize in the form of full value fish meal of fish offals from preliminary processing,
- Create the possibility for the usage of coagulates of protein and fat obtained in the post production sewage treatment for fodder or fertilizing possibilities.

The study was conducted as part of project number 5 P06G 01999/c4076

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L 49. EFFECT OF MODIFIED ATMOSPHERE PACKAGING WITH FREEZE-CHILLING ON SOME QUALITY PARAMETERS OF RAW WHITING, MACKEREL AND SALMON PORTIONS.

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Abstract

Modified atmosphere packaging (MAP) was combined with freeze-chilling to extend the shelf-life of raw whiting, mackerel and salmon fillets/portions. The MAP packs for mackerel and salmon (60% N_2 / 40% CO_2), and for whiting (30% N_2 / 40% CO_2 / 30% O_2) maintained their shape during freeze-chilling whereas packs with 100% CO_2 were slightly imploded with concave sides. The MAP samples had a shelf-life (in acceptability terms) of 5 (whiting and mackerel) and 7 (salmon) days compared with 3 and 5 days respectively for freeze-chilled fillets in air. The 30% N_2 / 40% CO_2 / 30% O_2 atmosphere performed best for the whiting fillets and the 60% N_2 / 40% CO_2 for mackerel and salmon.

Introduction

Multiples are pushing suppliers to innovate to allow them to grow their share of the lucrative fresh/chilled seafood market and eliminate the requirement for low-yield fresh seafood counters. Two methods which have considerable potential to extend the shelf-life of raw fish fillets are freeze-chilling and modified atmosphere packaging (MAP), and a combination of the two could have synergistic effects on product quality. Freeze-chilling involves freezing and frozen storage followed by thawing and retailing at chill storage temperatures (O'Leary *et al*., 2000). Freeze-chilling offers logistic and other advantages as foods can be prepared in bulk, frozen and stored at deep freeze temperatures until required (Fagan *et al*., 2003a). The objective of the current trial was to assess the suitability of freeze-chilling in combination with MAP for extending the shelf-life of raw whiting, mackerel and salmon portions. The inclusion of MAP should be beneficial in the chill storage phase (i.e. post-thawing) by inhibiting the natural spoilage organisms on the surface of the fillet thus extending the shelf-life.

Materials and Methods

Samples of raw whiting, mackerel and salmon were procured, sealed in atmospheres of 30% N_2 / 40% CO_2 / 30% O_2 (whiting), 60% N_2 / 40% CO_2 (mackerel and salmon) and 100% CO2 (whiting, mackerel and salmon), freeze-chilled, and tested after days 0, 3, 5 (whiting and mackerel) and 0, 5, 7 (salmon) at 4^{o}C as outlined by Fagan *et al*., (2003b). A range of sensory and physicochemical tests (Fagan *et al*., 2003a,b) were conducted and the results for some of these are presented in Tables 1-3. The results were tested by analysis of variance as two factors (atmospheres and test-dates) with four replicates. The results for atmospheres averaged over test dates are presented.

Results and Discussion

Sensory tests: Taste panel acceptability scores on a scale from 0 (unacceptable) to 6 (very acceptable showed no significant difference between the gaseous atmospheres after 5 (whiting and mackerel) or 7d (salmon). However, all treatments scored lower than the fresh samples for each species ($P< 0.001$; $P< 0.05$; $P< 0.001$) (Table 1). Free fatty acid (FFA) and peroxide values (PV) in the mackerel and salmon samples were low and did not influence taste panel response. Mackerel fillets packed in 100% CO_2 had the highest ($P< 0.05$) FFAs while salmon fillets in this atmosphere had the lowest ($P< 0.001$).

Table 1: Taste panel acceptability scores[a,b] for cooked whiting, mackerel and salmon portions subjected to modified atmosphere packaging with freeze-chilling

		Gaseous atmosphere					
Species	**Fresh[c]**	**Air[d]**	**30/40/30[e]**	**60/40[f]**	**100% CO_2[g]**	***F*-test**	**LSD[h]**
Whiting	3.93	3.46	3.50	-	3.18	P< 0.001	0.34
Mackerel	3.66	3.23	-	3.37	3.23	P< 0.05	0.32
Salmon	4.40	3.44	-	3.75	3.56	P< 0.001	0.61

[a] Scale from 0 (unacceptable) to 6 (very acceptable)
[b] For 4 replicates
[c] Tested on day 0 (no freeze-chilling or MAP)
[d] Stored in air for 5 (whiting and mackerel) or 7 (salmon) days
[e] Stored in 30% O_2 / 40% CO_2 / 30% N_2 for 5 days (only used for whiting)
[f] Stored in 60% N_2 / 40% CO_2 for 5 (mackerel) or 7 (salmon) days (not used for whiting)
[g] Stored in 100% CO_2 for 5 (whiting and mackerel) or 7 (salmon) days
[h] Least significant difference

TVC, TVBN, TMA: Samples packed in MAP had lower (P< 0.001) total viable counts (TVCs) (log_{10} cfu/g) than samples in air for raw fillets/portions of each of the three species (Table 2) and the 100% CO_2 atmosphere gave the lowest values. MAP had no effect on TVBN or TMA values in whiting and salmon portions, but mackerel fillets packed in air had higher TVBN (P< 0.05) and TMA (P< 0.001) levels than those from the other treatments. All TVBN values were within acceptable limits (95/149/EC).

Table 2: Effect of modified atmosphere packaging with freeze-chilling on the total viable count values (log_{10} cfu/g) of raw fish fillets / portions[a]

	Gaseous atmosphere					
Species	**Air**	**30/40/30**	**60/40**	**100% CO_2**	***F*-test**	**LSD**
Whiting	4.81	4.48	-	4.34	P< 0.001	0.20
Mackerel	4.88	-	4.18	3.99	P< 0.001	0.16
Salmon	6.23	-	5.04	4.53	P< 0.001	0.32

[a] See footnotes Table 1

Colour and texture: MAP had no effect on Hunter 'L' or 'a' values for whiting (means 51.2, -0.73), mackerel (43.0, 4.40) or salmon (41.4, 17.9) portions. Hunter 'b' values showed air-packed whiting (P< 0.05) and salmon portions (P< 0.01) to be less yellow than those in 100% CO_2. MAP treatments had no effect on the springiness of whiting and salmon portions, but mackerel fillets in 60% N_2 / 40% CO_2 were less springy (P< 0.01) than samples from the other treatments. However, the magnitude of the effect was small in practical terms. MAP treatment had no effect on the moisture content of whiting, mackerel or salmon portions and mean values were 80.6, 68.3 and 67.1g/100g respectively.

Gravity drip (GD) and centrifugal drip (CD): Fish portions in 100% CO_2 had higher GD than those in 30% O_2 / 40% CO_2 / 30% N_2 (whiting; P< 0.05) or in 60% N_2 / 40% CO_2 (mackerel; P< 0.001, salmon: P< 0.001). Samples packed in air had less GD than in the two MAP treatments. MAP had no effect on CD values in whiting. However, mackerel fillets packed in 60% N_2 / 40% CO_2 had less CD (P< 0.01) than those from the other MAP treatments. Salmon portions had low CD values in comparison with the other species (Table 3).

Table 3: Effect of modified atmosphere packaging with freeze-chilling on centrifugal drip values (%) of raw fish fillets / portions[a]

	Gaseous atmosphere					
Species	**Air**	**30/40/30**	**60/40**	**100% CO_2**	***F*-test**	**LSD[a]**
Whiting	11.0	13.9	-	12.4	NS	2.42
Mackerel	9.36	-	7.79	9.86	P< 0.001	1.21
Salmon	2.08	-	2.78	3.58	P< 0.05	0.69

[a] See footnotes Table 1

Conclusions

MAP with freeze-chilling is a suitable technology for raw whiting, mackerel and salmon fillets / portions and gives and extended shelf-life compared with freeze-chilling alone.

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L 50. FISH SURIMI AS AN INGREDIENT IN GLUTEN-FREE BREADS

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Abstract

A control gluten-free bread formulation based on rice and potato starch was supplemented with fish surimi (as a potential structure enhancer) at a 10% inclusion level (of starch weight). Frozen surimis from mackerel, blue whiting, red gurnard and pollock were evaluated. The inclusion of surimi darkened crust colour, and softened the crust and crumb except for bread with red gurnard surimi which had a much firmer crust and crumb, and the smallest loaf volume. There were inverse relationships between loaf volume x crust/crumb firmness and positive relationships between crust/crumb firmness x number of gas cells cm^{-2}. The breads with surimi had an attractive appearance. Paired comparison taste panel tests for acceptability indicated no difference between the control and the surimi breads, with the exception of bread with blue whiting surimi which was preferred ($P<0.05$) to the control.

Introduction

Gluten is the main structure-forming protein in flour. Its removal results in major technical difficulties for bakers and many gluten-free products exhibit poor mouthfeel and flavour characteristics (Gallagher, *et al.*, 2002). This prompted studies on a range of inclusions (as improvers) in gluten-free formulae including dairy proteins (Gallagher *et al.*, 2003a) and novel starches (Gallagher *et al.*, 2002) in breads, and fish surimi in fresh pasta (Kim *et al.*, 1990). The current study investigated the use of surimis of four different fish species as improvers in gluten-free breads made from a base of potato starch and rice flour. The breads were tested for loaf volume, number of gas cells cm^{-2}, crust/crumb colour and texture, and sensory acceptability.

Materials and Methods

Fish surimis: Frozen samples (supplied by IFREMER, France) of mackerel (*Scomber scombrus),* gurnard *(Aspitrigla cuculus),* blue whiting *(Micromesistius poutassou)* and pollock (*Pollachius pollachius)* surimi were added to a gluten-free bread formulation at 10% of flour weight. The frozen surimi samples had a moisture content of circa 80% (based on freeze-drying tests) and were tested for colour (freeze-dried samples; Minolta L*a*b*) and water holding capacity (WHC) (Gallagher *et al.*, 2003b).

Bread formulation and baking: A control (C) gluten free dough was prepared using a standard formulation (Gallagher *et al.*, 2003b) based on rice flour, potato starch and other ingredients. A second control (CW) was prepared with an equal water content to the surimi doughs. The latter were prepared using frozen surimis which were tempered overnight at $4^{o}C$, mixed with water and yeast to form a slurry, and added to the premixed dry ingredients. Mixing was continued followed by proofing, baking and evaluation of the loaves (Gallagher *et al.*, 2003b). Tests on the loaves included volume, texture profile analysis (after 24, 48 and 72 h; TAXT2*i*), crust/crumb colour (Minolta L*a*b*), image analysis, and sensory acceptability (Gallagher *et al.*, 2003b).

Results and Discussion

Bread colour and texture: The control gluten-free bread (CB) had the lightest crust colour of the six samples while the control bread with added water (CWB) had the lightest crumb colour (Table 1). The addition of extra water and/or fish surimi darkened crust colour. However, surimi type *per se* did not influence crust or crumb colour. This was unexpected as the freeze-dried blue whiting and mackerel surimis were darker than the pollock and gurnard samples (Minolta L*/b*: 2.95, 2.96, 4.18 and 4.59 respectively). However, the amount of surimi (on a dry matter basis) added was circa 2% of flour (rice flour + potato starch) weight and may have been insufficient to dull the white appearance of the starches in the bread crumb.

Table 1: Effect of fish surimi on the colour and texture of gluten free breads

Bread[1]	Colour[2]		Texture[3]	
	Crust lightness (L*)	Crumb lightness (L*)	Crust hardness (g)	Crumb firmness (g)
Control (CB)[4]	58	82	316	452
Control + water (CWB)	52	86	229	331
Mackerel (MSB)	53	82	217	177
Gurnard (GSB)	53	82	376	534
Blue whiting (BSB)	51	83	180	127
Pollock (PSB)	52	82	223	154
F-test	P<0.05	P<0.001	P<0.001	P<0.001
LSD	3.9	1.02	17.0	12.1

[1]See formula in Gallagher *et al.*, 2003b
[2]Minolta lightness values (L*); tested after 24 h
[3]TAXT2*i* texture analyser (g force); 24 h data
[4]Abbreviated terms for the breads

With the exception of the gurnard surimi breads (GSB), the addition of surimi gave a softer crust and crumb than the control breads (CB and CWB). This agrees with Kim *et al.*, who found that surimi addition reduced the firmness of cooked pasta. The firming effect of gurnard surimi may be due to its high water binding properties which result in more hydrogen bonding between the bread starches and fish and dairy proteins, and also a higher level of starch retrogradation. Water-holding capacity values for freeze dried surimi samples were 481 (gurnard), 382 (pollock), 326 (mackerel) and 306% (blue whiting). Overall staling patterns were the same for the four surimi breads in that crust hardness value decreased (249 vs 228 vs 195 g force; P<0.001) and crumb hardness values increased (248 vs 295 vs 350 g force; P<0.001) over days 1, 2 and 3 post-baking.

Loaf volume, gas cells, moisture content: BSB and MSB had much higher (P<0.001) loaf volumes than the other samples while GSB had the lowest volume. The PSB, GSB and CB samples had the smallest gas cells (P<0.01) and there was a negative relationship between loaf volume x no. of gas cells cm^{-2} [rank correlation coefficient (rcc = -0.55)]. There were also relationships (rcc) between loaf volume x crust firmness (-0.94), loaf volume x crumb firmness (-0.89), no. of gas cells cm^{-2} x crumb firmness (+0.31), and no. of gas cells cm^{-2} x crust hardness (+0.60).

Sensory acceptability: Paired comparison taste panel tests for acceptability between the control and surimi bread samples indicated that BSB samples were preferred to the control (15/5 preference ratio; P<0.05). Preference ratios for the other comparisons were 11/9 (MSB vs CB), 10/10 (PSB vs CB) and 8/12 (GSB vs CB). These data indicate that the panelists preferred the samples with the soft crust/crumb texture.

Conclusions

The results show that three of the surimis, gurnard surimi excepted, have potential as crust/crumb softeners in gluten-free breads. Breads with blue whiting surimi were preferred to the control in sensory tests.

Acknowledgements

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P 43. COLLABORATIVE TRIALS FOR THE ELABORATION OF A PROTOCOL FOR JOINT DETECTION OF PATHOGENIC *VIBRIO CHOLERAE* and *V. PARAHAEMOLYTICUS* IN SEAFOOD PRODUCTS

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Abstract

Because of recent changes in health regulations aimed at a better prevention of food poisoning outbreaks, food safety laboratories are now asked for routine screening of potentially pathogenic vibrios in fish products. There is a need for reliable and standardised methods of monitoring for these bacteria, and primarily *Vibrio cholerae* and *Vibrio parahaemolyticus*.

We selected and compared different protocols for the detection of this pathogenic vibrios in seafood products, with a view to proposing a single effective and normalised protocol usable in all laboratories in France and internationally for these two bacteria.

The following growth parameters were studied: the homogenate/seeded broth ratio [2 variants: 1/10 (w/w) and 1/100 (w/w)], the nature of the enrichment medium [2 variants: Alcaline Peptone Water, NaCl 1% and Alcaline Peptone Water, Yeast Extract, NaCl 2%], the enrichment incubation temperature (2 variants: 37°C and 41.5°C) and the length of the enrichment incubation (3 variants: 6 hours, 18 hours and 6 hours followed by subculture during 18 hours). Each protocol was applied in each laboratory to independent naturally contaminated samples of various geographical origins. Suspect colonies obtained after isolation of the enrichment medium on TCBS medium were identified by studying their biochemical characteristics, and this identification was checked by means of PCR. Pathogenic traits were then investigated by PCR.

Analysis of variance revealed the importance of the duration of enrichment incubation. *Vibrio parahaemolyticus* and *Vibrio cholerae* were best detected using an 18-hour incubation time. Six-hour enrichment and 6 hours followed by subculture during 18 hours gave significantly lower percentages of positive samples and could be excluded. Statistical analysis also showed that the subculture protocol was the least reproducible (significant difference between the two laboratories). In addition, a homogenate/seeded broth ratio of 1/10 (w/w) appeared better than a ratio of 1/100 (w/w).

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P 44. PRESTIGE OIL SPILL: PURSUIT OF CONTAMINATION LEVELS BY POLYCYCLIC AROMATIC HYDROCARBONS (PAHS) IN BIVALVE MOLLUSCS OF GALICIAN ESTUARY.

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Abstract

Galician coast (north west spain) has undergone very serious consequences after the disaster produced because of the 'Prestige' sunk on november 13th 2002: environmental impact and the fishery and aquaculture products contamination produced by the fuel spilled by the oil tanker, is the most serious disaster in galician community history.

Laboratories of different countries have studied the chemical composition of petroleum, the analyses relief that is basically composed of saturated hydrocarbons, resins and naphtalenes, and polycyclic aromatic hydrocarbons (PAHs) (CSIC). PAHs are a group of more than 100 lipophilic organic compounds, which confers a great stability and toxicity. Light fraction of PAHs tends to change throught time due to volatilization, solubilization and photo-oxidation phenomena. Nevertheless the heavy molecular weight of PAHs make these compounds scarcely biodegradable and displaying a high teratogene power (IARC). Due to great stability of this group of PAHs, an accumulation of them could take place in fish and seafood from the ecosystem. Concentrations reached in fishery and aquaculture products will depend on the concentrations in water and on the accumulation and metabolization speed of these compounds in marine organisms.

The aim of this work is to assess contamination levels after 'Prestige' disaster in mussels (*mytilus galloprovincialis*) farmed in areas of the galician stuary not directly afeccted by the oil spill. Since it is known that mussels need 7 months to depurate these PAHs in contaminated water, and approximately 2 months in clean water, an evolution-time study is of interest.

Conte`s rapid method for PAHs determination in vegetable oils, has been optimized for fish and bivalve molluscs. It is based on solid phase extraction of fat sample in hexane, and HPLC analysis, with spectrofluorimetric detection.

We have studied 6 heavy PAHs to know the safety of mussels and the environmental impact of the spill in the galician coast.

Introduction

The sunk of the oil tanker named "Prestige" on November 13th 2002, was the greatest catastrophe affecting to the spanish sector of the fishery and aquaculture products. Then, 25,000 tons of fuel were spilled to the sea, whose spot has practically included the totality of the Galician coasts, except the inner areas of the stuaries ("rías"). In addition to the environmental impact caused by the fuel, Polycyclic Aromatic Hydrocarbons (PAHs) could also contaminate fishery and aquaculture products.

Chemical composition of the fuel spilled by the Prestige was reported to be 19% of saturated hydrocarbons, 35 % of resins and naftalenes, and more of a 46% of aromatic hydrocarbons, with a total concentration of Polyclic Aromatic Hydrocarbons (PAHs) about 400 mg/Kg. PAHs are a group of compounds having more than 4 aromatic rings that confers them great stability, bioaccumulative behavior and a significant carcinogenic and teratogen activity (SCF, 2002). In this sense, the most toxic compounds are Benzo (a) Pireno and Dibenzo (a,h) Anthracene (EPA, 1993), but at least 6 compounds are classified as 2A "probably carcinogenic" or 2B "posibly carcinogenic" by the IARC: Benzo(a)anthracene, benzo(b)fluoranthene, benzo(k)fluoranthene, benzo(a)pyreno, dibenzo(a,h)anthracene, benzo(g,h,i)perilene. All of the shows low biodegradability and volatility, being this fraction a good monitoring parameter to pursuit an accidental contamination by hydrocarbons.

It is known that bivalve mollusks are specially prone to contamination because their enzymatic detoxification pathways are less developed than in vertebrates, and also because they are sedentary organisms that filter a great amounts of water. Crustaceans are not easily contaminated although they bioaccumulate PAHs in higher rate than fishes.

Maximum or threshold values of the 6-PAH content in fish and shellfish following an oil spill previously stablished by the French Agency of Food Safety (AFSSA) after the "Erika" spill, were later assumed by the Spanish Agency of Food Safety in the "Prestige" episode:

- 0,2 mg/kg dry weight for bivalve mollusks and crustaceans
- 0,02 mg/kg dry weight for fish.

Here are reported the observed values of the 6 “carcinogenic” PAHs in bivalve samples collected before and after the Prestige spill. Samples collected after the spill were from the inner side of the stuaries (“rias) considered “free of fuel” by the spanish health authorities at the moment of the sampling.
The aim of this work was:

- To compare the content of PAHs in the bivalves before and after the spill.
- To evaluate the “safety” of the shellfish extracted after the spill from “free of fuel” areas.
- To monitor the temporal evolution of PAH levels in marketed bivalves, from the spill date to present.

Materials and Methods

28 bivalve samples were collected in 13 different sampling points along the galician coast before the Prestige spill and 49 bivalve samples from 16 sampling points considered “free of prestige fuel” by the spanish health authorities after the spill, were analyzed for their PAH content.

6 PAH compounds were determined by HPLC with spectrofluorimetric detection: benzo(a)anthracene, benzo(b)fluoranthene, benzo(k)fluoranthene, benzo(a)pyreno, dibenzo(a,h)anthracene and benzo(g,h,i)perilene.

Rapid clean-up of the organic extract from freeze-dried samples was carried out by Solid Phase Extraction on large silica cartridges previous to HPLC determination The analytical procedure was developed in validated in our lab (Sobrado, 2003).

Results and Discussion

Results obtained in this study are summarized in table 1, reflecting the sum of 6 PAHs before and after the “Prestige” sunk. According to these data, 19,3 µg/Kg dry weight is the maximum value obtained in bivalve molluscs samples captured before spill, while almost 100 µg/Kg is the maximum concentration determined in bivalve samples collected after the spill in “free of fuel” waters. In spite of this value, 5 times higher, it does not imply a contamination due to the spill; in fact, this value is half of the maximum concentration for bivalve molluscs established by AFSSA (200 µg/Kg).

There are several reports, in which values refered to the sum of 16 PAHs in *Mytilus galloprovincialis* samples, have been published: in stuaries with healthy conditions, levels from 25 to 390 µg/Kg dry weight were obtained (Baumard and others, 1998[1], and Baumard and others, 1998[2]). These values are lower than the equivalent reference value established by AFSSA (500 µg/Kg dry weight).

Our results show that bivalves marketed after the Prestige spill and extracted from esturine waters considered “free of fuel” was free of significant PAH contamination, although PAHs concentrations higher than usual were found in several samples.

Monitoring of the PAHs evolution in time showed an increment of PAHs concentration during months December 2002 and January 2003, and from March to date, levels of hydrocarbons decreased again (Figure 2).

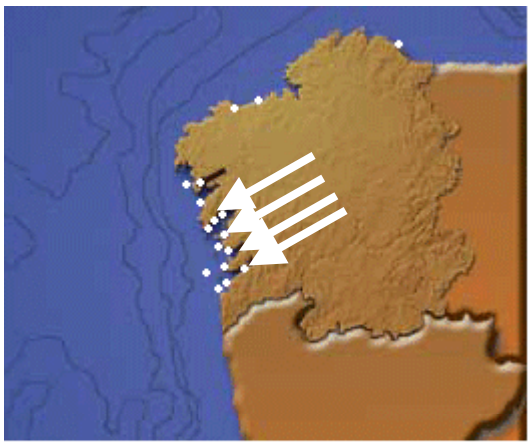

Figure 1. Sampling points are show with dots. Arrows indicate the estuarine waters (“rias”) free of fuel deposition.

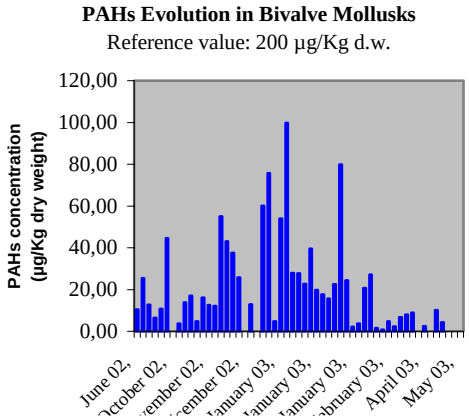

Figure 2. Trend of PAH levels in bivalve mollusks from june 2002 to may 2003. Samples captured in 17 different points of gallician coasts.

Table 1 –Concentration ranges of 6 PAHs (µg/Kg dry weight) in samples analyzed before and after "Prestige" sunk.

Species	PAHs (sum) (µg/kg d.w.) (Before "Prestige")	**PAHs (sum) (µg/kg d.w.) (After "Prestige")**
Mussel	**n.d. – 19.3 (n= 8)**	n.d. – 99.9 (n= 29)
Cockle	n.d. – 3.5 (n= 2)	n.d. (n = 1)
Clam	n.d. – 3.3 (n= 4)	n.d. – 4.3 (n = 2)
Others	n.d. (n= 14)	n.d. – 6.8 (n= 17)

Figure 3. Comparison of median, quartile and maximum values of PAH content in bivalves marketed before and after the Prestige spill

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P 45. DETERMINATION OF HEAVY AROMATIC POLYCYCLIC HYDROCARBONS IN FISH PRODUCTS.

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Abstract

Polycyclic aromatic hydrocarbons (PAHs) are considerated persistent organic compounds, because they could be long time in the environment without changing their toxical properties. A common characteristic of these compounds is poor water solubility in addition to their lipophilic properties. Thereby, they are bioaccumulated in the food chain, specially in fishery products.

To develop a simple quantification method for hydrocarbons in food matrix is a topical of interest to carry out routine analysis, since sample preparation is laborious and time-consuming.

We have developed a PAHs determination process in fishery products based on the method of Conte and Moret for polycyclic aromatic hydrocarbons in vegetable oils. Fat is extracted from 2 g of dehydrated sample, through a chromatographic column with a mixture of pentane : dichloromethane (1:1). The sample is dissolved in 10 mL of n-hexane and 1 mL is loaded onto a 5 g silica cartridge, elluting PAHs fraction with n-hexane : dichloromethane (70:30). After solvent evaporation, the residue is redissolved in acetonitrile and is injected in HPLC with C18 phase reverse column and a spectrofluorometric detector.

Results obtained in different fishery products, show a recovery rate between 70 and 125%. In addition, statistical studies demonstrate that recovery of the method is independent on the sample matrix.

Introduction

Polycyclic aromatic hydrocarbons (PAH's) constitute an organic compound family well-known due to their toxical properties. They are formed by natural or anthropogenic processes by the incomplete combustion (pyrolysis) of organic compounds of high molecular weight causing molecules of minor weight, which are volatile in gaseous phase [1].

Due to its lipophilic characteristic and the high resistance to chemical and biological degradation, PAH's are bioaccumulated in the food chain. Moreover, they are hydrophobic compounds, establishing a high risk of contamination in marine organisms. Specially in bivalve molluscs, because they can accumulate hydrocarbons of high molecular weight, due to they filter big amounts of water, and they are not able to metabolise [2].

Many methods of analysis have been described for PAH's detection and quantification, but most of them are very laborious and time-consuming[3]. Therefore, it is necessary to develop a simple and rapid analytical procedure for fishery and acuiculture products, allowing the possibility to carry out routine and control analyses.

In this work, a simple method has been developed for polycyclic aromatic hydrocarbons quantification in fishery matrix based on Conte and Moret method for PAH's determination in vegetable oils[1].

Experimental

Sampling: All fishery products were come from Galician coast

Reagents: Hexane, Dichloromethane, Pentane (Suprasolv grade, Merck); Sodium Sulphate (for column chromatography, Merck); Silica gel 60 (0.063-0.200mm, Merck); Acetonitrile (gradient HPLC grade, J. T. Baker) and Milli Q Ultrapure Water. Polycyclic aromatic hydrocarbons standard has been distributed by Dr. Ehrenstorfer GMBH: Benzo (a) Antracene (99.0 %); Benzo (b) Fluorantene (99.5 %); Benzo (k) Fluorantene (99.5 %); Benzo (a) Pyrene (99,5 %); Dibenzo (a, h) Antracene (99,5 %) and Indene (1,2,3-c,d)Pyrene (99,0 %).

Preparation of standards: stock solutions containing 10 mg/mL of each standard and were prepared in acetonitrile. A PAH's standard mixture (20 ng/mL) solution was prepared from stock solutions in acetonitrile and was stored at room temperature in amber volumetric flask to avoid possible light degradation. Calibration solutions were prepared by appropiate dilutions of the standard mixture.

Apparatus: For lyophilization of fishery products, a Telstar Cryocool 40 freeze-drier was used. Solvent evaporation was carried out in a Laborota 4000 connected to a Büchi V-503 vacuum pump. To clean solid phase extraction cartridges, a Manifold J.T. Baker, spe 12-G, was used. Detection and quantification was carried out on a HPLC Hewlett Packard 1100 equipped with a quaternary pump, a Chromspher PAH (Chrompack) reversed-phase column (3 µm, 100 x 4,6 mm I.D.) and a Hewlett Packard 1100 spectrofluorimeter. For adquisition and processing spectral information a Hewlett Packard " HP Chemstation " was used.

Procedures

1. *Extraction and Clean-up*

20 g of sample were lyophilised. Then, fat was extracted from 2,0 g (dry mass) in a chromatographic column (26 cm x 1,2cm ID), lyophilised sample with 4 g of anhidre sodium sulphate, using 100 mL of pentane:dichlorometane (1:1). Solvent mixture was evaporated to dryness and the extract was re-dissolved in 10 mL of hexane. 1 mL of the sample was loaded on a 5 g silica cartridge, previously conditioned with 20 mL of dichloromethane, dried completely under vacuum and 20 mL of hexane. PAH's were eluyed with a mixture of hexane: dichloromethane (70:30). The first 8 mL were discarded, collecting the following 8 mL. The sample was evaporated to dryness under nitrogen stream and the residue is re-dissolved in 250 µL of acetonitrile.

2. *Chromatographic Method*

10 µL aliquot of acetonitrile solution is injected into the HPLC. The mobile phase consisted of acetonitrile and water at a flow rate of 1,2 mL/min. The gradient elution program started with 50% water and 50 % acetonitrile, (during 3 minutes) going to 100 % of acetonitrile in 30 minutes, (isocratic for 2.50 minutes). The emission and excitation wavelengths have varied with each compound. The required conditions are the following:

Compuesto	λ_{exc}	λ_{em}
Benzo (a) Antracene	***270***	***390***
Benzo (b) Fluorantene	***260***	***430***
Benzo (k) Fluorantene	***256***	***410***
Benzo (a) Pyrene	***256***	***410***
Dibenzo (a,h) Antracene	***300***	***418***
Indene (1,2,3-c,d) Pyrene	***300***	***418***

3. *Confirmation of Results*

The analite identity is confirmed by comparing excitation and emission spectra between chromatographic sample peak and standard. Excitation spectra have to be acquired by wavelengths sweep from 230 to 380 nm and the emission spectra from 300 to 500 nm. The peak is confirmed when the emission and excitation spectra of both should be at least 700 per 1000. Besides, maximum wavelengths of both (emission and excitation spectra) between the standard and the sample is ± 5nm.

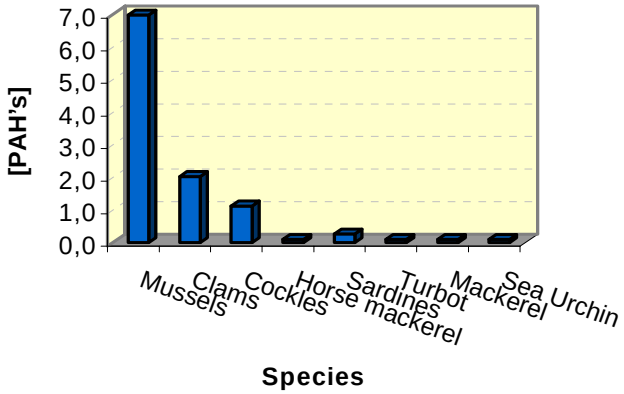
Results and Discussion

The efficiency of the method has been studied in diverse fishery product matrix. Eight species have been chosen: mussels, clams, cockles, horse mackerel, sardines, turbot, mackerel and sea urchin; grouped in bivalves, fishes and equinoderms.

It has been obtained a quantification limit of 0,6 ppb on the dry residue. In wet samples, quantification limit value is different in each sample depending on the amount of water. In the bibliography have been reported similar values for fishery products, using different analytical procedures[4].

The repeatability of the method was tested on the eight different matrix. The relative standard deviation (R.S.D.) calculated for the worked range is lower than the Horwitz R.S.D. in all the species.

The recovery percentage has been calculated for each specie, adding two different concentrations of PAH's standard mixture. It has been calculated recovery values between 70 and 125 %. Recoveries values have allowed to carry out a statistical study. It has been demonstrated the recovery of every hydrocarbon is independent of the sample matrix. Besides, it has been observed that the behavior of the polycyclic aromatic hydrocarbons in the same matrix is similar, because there is not significatly difference between PAH´s for each specie.



Finally it is necessary to emphasize, that the obtained results are in conformity with those found in the bibliography[2]. Polycyclic aromatic hydrocarbons concentration is major in bivalve molluscs, specially in mussels, because they filtered great amounts of water and they can not metabolise all PAH's accumulated. Nevertheless, in the fish and equinoderms, the concentration is very low being in the majority of the samples low than the limit of quantification.

Conclusion

We have developed a simple method for isolation and purification PAH's in different fishery products. The lipid extraction process has been used previously in other organic contaminants, such as dioxins and furans, because is a rapid and simple method and has been obtained very satisfactory results[5]. The clean-up stepwise is based on Conte and Moret procedure for PAH's determination in olive oil, being necessary to optimize some parameters due to the differences in the sample matrix.

Repeatability and Recovery data demostrate that the method is adequated to analyse polycyclic aromatic hydrocarbons in the studied species. Moreover, the statistical study reveals that the PAH's probably will have the same behavior in other products fishery.

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P 46. MOLECULAR CHARACTERISATION ON *LISTERIA MONOCYTOGENES* STRAINS ISOLATED FROM COLD SMOKED SALMON PLANT AND COOKED PEELED SHRIMP *(PANDALUS BOREALIS*) PLANT IN ICELAND.

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Abstract

Listeria monocytogenes is commonly found in the environment and has been isolated from various types of food. Surveys at IFL over 5 year period in cold-smoked salmon plant and two cooked peeled shrimp plants show that *L. monocytogenes* is found widely in the processing environment. Strains were typed using Pulsed-Field Gel Electrophoresis (PFGE) and the results indicate that *L. monocytogenes* is an persistent in-house flora in these plants.

Introduction

Listeria monocytogenes is commonly found in the environment and has been isolated from various types of food. In recent years attention has been focused on *L. monocytogenes* as a cause of food borne illnesses. Infection with *L. monocytogenes* may result in very severe manifestation for human health, with a high mortality rate, especially for pregnant women, neonates, immuno-compromised people and the elderly. Invasive listeriosis is a realtively rare but severe disease with incidence rates typically of about 4-8 cases per million indivituals and fatality rates of 20-30% among hospitalised patients. In 1978-1997 the incidence rates in Iceland were 8,3 cases per million indivituals and fatility rare 33%.

Moreover, the contamination of raw or processed food products by this micro-organism may lead to drastic economic losses for the food industry. This pathogen is cold tolerant and is therefore of great concern in ready to eat foods. With increased consumption of ready-to-eat foods, authorities have requested more surveillance.

Materials and Methods

Pulsed-Field Gel Electrophoresis: *L.monocytogenes* strains were inoculated onto Tryptic Soy agar plates and incubated over night at 35°C. A single colony was inoculated into a Brain Heart Infusion broth and incubated overnight at 35°C, 2 ml of the broth was washed in 5 ml of PIV buffer (10mM Tris, 1M NaCl) the cells were harvested by centrifucation at 2500 rpm (EconoSpin, Sorvall Instruments, USA) for 15 min. at 4°C. The DNA isolation was performed as described by Maslow et al. with the following modification, instead of insert molds a syringes were used. The plugs were lysed for only 4 h. Only a single overnight was at 50°C was used. 5U AscI and 10U ApaI (New England Biolabs, Beverly, Mass) were used for restriction endonuclease digestion. The samples were electrophresed through 0.9% and 1.0% (w/v) agarose (SeaKem GTG, FMC Bioproducts, Rockland, Maine), respectively, in 0.5 x Tris -borate EDTA (45 mM Tris, 4.5 mM boric acid and 1 mM sodium EDTA) at 210 and 200V, respectively at 14°C in CHEF DRIII system (BioRad Laboratories, Richmond, Cal). The pulse times ramped from 1s to 28s for 10 h and 28s to 30 s for 10 h and 1 s to 18 s for 20 h, respectively. The gels were stained with ethidium bromide and photographed under UV transillumination using GelDoc 2000 documentation system (BioRad Laboratories). The photos were saved as TIFF-files. Lamda ladder PFG marker (New England Biolabs) was used for fragment size determination.

Analsyis was performed visually as well as GelComparII (Applied Mathd, Kortrijk, Belgium) was used to copmpare strains. Any difference between two PFGE profiles was considered sufficient to distinguish these profiles.

Results and Discussion

Surveys: Work at Icelandic Fisheries Laboratories (1997-2001) in processing of cold-smoked salmon and cooked peeled shrimp has shown that *Listeria* is found widely in the processing environment. Over the past 5 years there have been several surveys done in the processing environment of cold-smoked salmon and cooked peeled shrimp. In the cold-smoked salmon industry there have been taken 86 raw material samples where *Listeria* spp was found in 22.1% and *L. monocytogenes* in 16.3%, 436 samples have been taken from the processing environment with 16.7% positive for *Listeria* spp and 12.2% *L. monocytogenes* and 117 samples from the final product with 5.1% positive for *Listeria* spp and 3.4% *L. monocytogenes*. In the cooked peeled shrimp industry there have been taken 45 raw material samples with 20% positive for *Listeria* spp and *L. monocytogenes*, 568 samples taken from the processing environment with 13% positive for *Listeria* spp and *L. monocytogenes* and 35 samples taken from the final product but none were positive for *Listeria*.

These results indicate that *Listeria* is widely distributed in the raw material and the processing environment but the incidence is low for the final product.

PFGE analysis: All *L. monocytogenes* strains (3 strains from each sample) isolated in these surveys have been typed using Pulsed-Field gel electrophoresis (PFGE), using two restriction enzymes, AscI and ApaI. One cold-smoked salmon plant (Plant A) were mainly surveyed and there seem to be the same clone persistant in the processing environment since at least 1997. Out of 129 strains isolated in plant A, 118 were same PF type (PFGE type) using AscI, these samples were taken from the raw material, the processing line before and after cleaning and the final product. In a company (Company A)that processed further smoked salmon from plant A the same clone was found in 28 strains (out of 38 strains isolated there). One strain from the fish farm that produced salmon for plant A were also of the same PF type. This indicates that the same clone seems to be persistant in the plant and might originally have come into the plant from the fish farm.

Two cooked peeled shrimp plants were mainly surveyed (Plant B and Plant C), in plant B there was one PF type dominating 88 strains out of 104 strains and in plant C another PF type dominated were 41 strain out of 73 belonged to it (using AscI). Ten of the 38 strains isolated from company A belonged to the same PF type that was dominating in plant C, but to our knowledge product from plant C were not processed there, but the plant and the company are both located in the same area.

Conclusions

These results indicate that *Listeria* is established as an in-house flora in cold-smoked salmon and cooked peeled shrimp plants in Iceland at least since 1997.

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P 47. THE OCCURRENCE OF *LISTERIA MONOCYTOGENES* FOR COLD SMOKED AND GRAVAD FISH IN THE ICELANDIC RETAIL MARKET

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Abstract

A survey of commercially available brands of vacuum-packed cold smoked and gravad salmon and trout was conducted in Reykjavik, Iceland. A total of 38 packages of fish from 10 different companies were sampled to detect and quantify the occurrence of *L. monocytogenes* in smoked and gravid fish at the retail market. The samples were tested for temperature at the display cabinet, water activity (Aw), pH value, total aerobic plate count (APC), total and faecal coliforms and enumeration and detection of *L. monocytogenes*. Results were used to determine the remaining shelf life of the product using the Pathogenic modeling program (PMP version 5.1) in those *L. monocytogenes* positive samples. Approximately 47 and 3% of samples were outside specifications for APC and *L. monocytogeenes* counts respectively. *L. monocytogenes* was found in 13% of the samples and gravad salmon samples shown the highest incidence (28%). The AW and pH values were lower for wood-smoked trout samples. Therefore, the combination of low pH and Aw for wood smoked trout WST may contribute to keep this bacteria at non detectable levels if stored below 5°C. About 40% of the positive samples were temperature abused. The amount of *L. monocytogenes* found in cold smoked or gravad products is not high, but temperature abuse can stimulate its growth. When comparing the producer date stamp of positive samples with the predicted shelf life, only the gravad salmon temperature abused samples (13.2°C) were unacceptable. The rest of the positive samples were not temperature abused, and the producer date was shortest than the predicted, regardless of the initial population. This shows the importance of good storage conditions. Cold smoked and gravad salmon and trout can be considered a safe product to eat when processed under good manufacturing practices and stored at low temperatures.

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P 48. BACTERIAL COMMUNITY STRUCTURE OF COLD-SMOKED SALMON USING A PCR-TTGE TECHNIQUE

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Abstract

Bacterial community structure of cold-smoked salmon is generally estimated by culture dependant techniques. Molecular-based community analysis are increasingly employed in the related fields such soil microbiology and have provided a new insight into microbial diversity. In this way, we thought that PCR-TTGE could be an interesting tool to monitor the microbial dynamic and evolution of bacterial community structure and its involvement in spoilage process. This technique is based on the electrophoretic separation of PCR-amplified double strand DNA under denaturing conditions (achieved by temperature gradient).

The aim of this study was to assess the bacterial community structure on the cold-smoked salmon. To study the bacterial changes in cold-smoked salmon we have chosen to amplify rpoB gene fragment. Like 16sDNA, the rpoB gene is present in all bacterial species, but advantageously as a single copy gene. A Nested-PCR technique, using degenerated non-clamped primers in first PCR and non degenerated clamped primers in the Nested-PCR, was used for application on TTGE profiles. rpoB amplification and TTGE profiles were obtained for all the pure strains tested which are generally isolated from cold-smoked salmon.

Extraction of indigenous fish bacteria by various protocols was compared on the bias of PCR-TTGE profiles. Profiles obtained by PCR-TTGE technique showed various different bands that could be associated to pure strain profile.

First results obtained from this study must be optimised but open the way of monitoring changes of bacterial community structure in fish products.

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R 2. CHEMICAL AND MICROBIOLOGICAL CHANGES OF SARDINE (*SARDINA PILCHARDUS*) IN MODIFIED ATMOSPHERES

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Abstract

The effects of modified atmosphere packaging (MAP) and vacuum packaging (VP) on sensory, chemical, and microbiological changes in sardine with emphasis on the quality and safety parameters were investigated. Quality assessment of sardine in VP and MAP for up to 15 days was evaluated by the monitoring of sensory quality, total viable counts (TVC), nucleotide degradation products, histamine, trimethylamine (TMA), total volatile base nitrogen (TVB-N). The observed shelf life of sardine was found to be 12 days in MAP, 9 days in VP and 3 days in air. Bacteria grew most quickly in sardine stored in air, followed by those in VP and the lowest counts were with MAP. The concentration of histamine increased and its level reached over 20 mg/100g for fish stored in air, 13 mg/100g for VP and 10 mg/100g for MAP at 15 days. The highest concentration of TMA was obtained from sardine stored in air, followed by sardine stored in VP and the lowest in MAP. The formation of TVB-N increased with time of storage. When the TVC had reached 10^6cfu/g, the TVB-N content was approximately found 15 mg/100g muscle for all storage conditions. Storage of sardine under modified atmosphere conditions decreased the production of ATP derivatives, inhibited bacterial growth, reduced the formation histamine, TMA and TVB-N and extended sensorial rejection. Subsequently, self-life was prolonged and quality was maintained.

Functional properties of fish components.

Lectures 51-63
Posters 49-57

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L 51. HEMOGLOBIN-MEDIATED OXIDATION OF WASHED COD MINCE PHOSPHOLIPIDS: EFFECT OF PH AND HEMOGLOBIN SOURCE

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Abstract

Hemoglobin (Hb) is regarded a major catalyst of lipid oxidation in fish muscle during post harvest storage. Deoxygenation and autoxidation of Hb appear to accelerate the lipid pro-oxidative properties. The physicochemical properties of Hb differ between different species, which could explain why certain fish are more susceptible to rancidity than others. Deeper knowledge on this topic could lead to successful design of species-specific antioxidative strategies for use in the fish industry.
This study was carried out to compare the pro-oxidative properties and the deoxygenation/autoxidation patterns of Hb´s from white-fleshed fish (Winter flounder, Atlantic pollock) and migratory dark fleshed fish (Atlantic mackerel, menhaden). It was also studied whether these properties were affected by slight acidification, something that occurs naturally in the muscle of many fish species *port mortem*.
Hemolysates from the four species were added to a washed cod mince model system adjusted to pH 6 and 7. During ice storage, development of TBARS and painty odor was followed. Hemolysates were also added to an aqueous phosphate buffer model system (pH 6 and pH 7) in which storage induced Hb deoxygenation and autoxidation were followed spectrally.
At pH 6, all four Hb´s were highly and equally active as pro-oxidants, while at pH 7, their pro-oxidative activity ranked them as follows: pollock > mackerel > menhaden > flounder. Pollock Hb was the only Hb unaffected by the change in pH, all others became less catalytic as the pH was rasied. The high catalytic activities of the Hb´s at pH 6 corresponded with high formation of deoxy-Hb and met-Hb. Among the four species, pollock had the most extensive formation of deoxy- and met-Hb at both pH's, which could explain its high catalytic activity. The differences between the other hemoglobins at pH 7 could not be explained only from deoxygenation and autoxidation reactions. This indicates involvement of other structural differences between the Hb´s or differences in the content of low molecular weight compounds of the hemolysates.

Introduction

Lipid oxidation is a major reaction causing quality losses in fish muscle during storage. Currently, considerable attention is given hemoglobin (Hb) as one of the most potent lipid oxidation catalyst in *post mortem* fish muscle. Hb can become mixed with oxidation substrates of the muscle as a result e.g. of mechanical treatments (filleting, grinding), washing, and oxidative destruction of the erythrocyte membranes. Hb can catalyze oxidation via several mechanisms, (i) by decomposing pre-formed lipid hydroperoxides into lipid free radicals, (ii) by forming oxygen free radicals via its own autoxidation, (iii) by becoming converted to the hypervalent ferryl-Hb radical and (iv) by acting as a pseudo-lipoxygenase enzyme (Kühn *et al*., 1981, Everse & Hsia, 1997; Griffon *et al*., 1998).

In vitro, pH-reductions equal to those naturally occurring in certain *post mortem* fish muscle; e.g. from pH 7 to 6.2, have been shown to increase the pro-oxidative activity of Hb from trout (Richards & Hultin, 2002; Undeland *et al*., 2001). It was thought that the production of deoxy-Hb occurring in response to acidification (the Bohr effect) was involved, as deoxy-Hb better exposure of the catalytic heme group/Fe-atom (Levy & Rifkind, 1985) and more easily gets oxidized (Shikama, 1998).

Since different fish species operate under greatly different physicochemical conditions, their Hb´s differ largely, both in conformation and in response e.g. to pH and temperature changes (Weber, 2000). Hb from herring and mackerel were previously found to be more pro-oxidative toward washed cod mince lipids than trout Hb (Richards & Hultin, 2003). It was hypothesized that the migratory lifestyle of the two pelagic species called for Hb´s that rapidly released oxygen upon lactic acid production, and thus, that easily become convert into the tentatively more pro-oxidative deoxy-Hb form. Deeper knowledge on this topic could lead to successful design of species-specific antioxidative strategies for use in the fish industry.

The aim of this study was to compare the ability Hb´s from four different fish species to catalyze oxidation of washed cod mince lipids during ice storage. Two species were migratory pelagic species (Atlantic mackerel and menhaden) and two non-pelagic whitefish species (Atlantic pollock and Winter flounder). To elucidate the role of pH for the catalytic properties, storage studies were carried out at pH 6 and 7. To be able to explain pH- and/or

species related differences in the pro-oxidative activity of the Hb´s, storage induced Hb-autoxidation and deoxygentation in an aqueous model system was also studied.

Materials and methods

Bleeding of fish and preparation of hemolysate. The four fish were bled as described by Rowley (1990). Hemolysates were prepared from the whole blood according to Fyhn et al. (1979). Hb-levels in the hemolysates were measured with the method of Brown (1961) as adapted by Richards and Hultin (2000).

Washed cod muscle model system. White cod muscle was ground and washed according to Richards and Hultin (2000) with the modification that the last wash in 50 mM phosphate buffer was done either at pH 6 or pH 7 instead of at pH 6.7. The final washed cod was frozen at (-80°C).

*Preparation of oxidation system and analysis of oxidation***:** Thawed washed mince was manually mixed with 200 ppm streptomycin to prevent bacterial growth. Half of the mince was adjusted to pH 7 and half to 6, and each batch was then divided into five 30 g-portions. In four of them, lipid oxidation was started by manually mixing in the four different fish hemolysate to final Hb-levels of 5.8 µmol/kg mince. In controls, the hemolysates were replaced by distilled water. The samples were flattened out in the bottom of screw-capped Erlenmeyer flasks and were stored on ice for up to 7 days. At regular intervals, 3-4 trained panelists sniffed the headspace above the samples by uncapping the 225 mL screw-capped Erlenmeyer flasks. Panelists concentrated on detecting painty odors using a scale of 0 to 10, with 10 being the strongest. At regular intervals, 1g-sample "plugs" were also taken out from the washed cod model system and were frozen in aluminum foil at –80°C. Follwoing completed sampling, TBARS were analyzed after TCA-extraction of the "plugs" (Lemon, 1975). The lag phases for development of painty odor and TBARS are defined as time elapsing until an intensity of 1 and a value of 10 µmol MDA/kg tissue, respectively, are reached.

Hemoglobin oxygenation and autoxidation. The four hemolysates were added to 50 mM sodium phosphate buffers at pH 6 and pH 7 to give 10 µM Hb/mL. Buffer samples were stored in test tubes on ice, and were scanned daily at 2°C from 500-630 nm against a buffer blank. Relative oxygenation was calculated as: relative oxygenation = (Absorbance @ 575 nm- Absorbance @ 560 nm)*1000. Hb-autoxidation was measured as a reduction in the absorbance peak at 575 nm using the following formula (Shikama, 1998): -ln (A_{575}(stored sample)/A_{575}(unstored sample))

Results

At pH 6, painty odor developed without lag phase in washed cod mince samples containing pollock Hb. Mackerel, menhaden and flounder Hb´s gave rise to ~0.2 days lag phase. The rate of paintiness increase was slower for flounder Hb than the other Hb´s. At pH 7, painty odor induced by mackerel, menhaden and flounder Hb´s was slower compared to at pH 6, while pollock Hb gave rise to the same fast painty odor development as at pH 6. The length of the sensory lag phases at pH 7 ranked the catalytic power of the Hb´s as: pollock (0 day) >mackerel (1 day) >menhaden ~ flounder (2.7 days).

TBARS values also developed very quickly at pH 6, with virtually no lag-phases observed for any of the Hb´s. Mackerel Hb catalyzed TBARS formation the fastest. At pH 7, TBARS development was significantly slowed down for all Hb´s except in the case of pollock. Based on TBARS lag phases, the catalytic power of the Hb´s at pH 7 were ranked as: pollock (0 day) >mackerel (0.8 day) >menhaden (2.5 days) >flounder (3.8 days). The controls with no added hemoglobin did not change noticeably in odor or TBARS on storage.

At time 0, all four Hb´s were relatively less oxygenated at pH 6 compared to at pH 7. At pH 6, menhaden Hb was less deoxygenated than the other three Hb´s which showed similar values. At pH 7, pollock Hb stood out in that it was more deoxygenated than the other three Hb´s. Hb-autoxidation followed a first-order reaction, particularly at pH 6. In the initial fast phase, the Hb´s autoxidized as: pollock > flounder ~menhaden >mackerel. At pH 7, autoxidation proceeded considerably slower than at pH 6. Based on the changes during the fast period, the Hb´s were ranked as: pollock > mackerel~menhaden~flounder.

Discussion

That washed cod mince containing mackerel, menhaden and flounder Hb´s oxidized faster at pH 6 compared to at pH 7 was in accordance with previous results from studies of trout Hb-mediated oxidation of washed cod mince (Richards & Hultin, 2000; 2002; Undeland *et al*., 2001). The finding can probably be explained by the higher levels of deoxy-Hb (the Bohr effect) and the subsequently faster conversion to met-Hb at pH 6 than at pH 7. Deoxy-Hb and met-Hb both have structural and reactive properites that could explain why they are stronger pro-oxidants as compared to reduced oxy-Hb (Levy & Rifkind, 1985; Shikama, 1998). It is possible that the fast met-Hb formation at pH 6 was preceeded by acid-induced dissocitaion of tetrameric Hb into Hb-dimers/monomers (Griffon *et al.,* 1998). That pollock Hb was as pro-oxidative at pH 7 as at pH 6 was probably connected to its relatively high degree of deoxygenation and autoxidation also at neutrality. The reduction from pH 7 to pH 6 affected deoxygenation and autoxidation of pollock Hb less than it affected the other Hb´s. Possibly, the relative amounts of deoxy and/or met-Hb present at pH 7 might already have exceeded the levels where they were limiting for carrying out oxidation of the washed cod mince lipids. This could indicate that pollock Hb has its Bohr effect located well above pH 7, and thus, that a higher pH is required for reaching a stage where the level of deoxy and/or met-Hb is low enough for lipid oxidation to be slowed down.

At pH 7, but not at pH 6, all four Hb´s were clearly differentiated in terms of their pro-oxidative activities. They were ranked as: pollock >mackerel> menhaden >flounder. Except for in the case of pollock, this order was not reflected in the levels of deoxy-Hb and met-Hb at pH 7. The latter ranked the species as: pollock > mackerel ~ menhaden ~ flounder. We therefore believe that the species differences between Hb´s were rather due to the different "life styles" of the studied fish. While menhaden and mackerel are migratory, and flounder slow-swimming, pollock swims in burst. The latter could explain why very quick oxygen off-load would be needed in pollock Hb at any sign of acidification. The fact that pollock was the studied species living at the largest depth and thus, in the coldest waters, could also be important. It is known that fish heme proteins have a more flexible and unstable protein conformation possibly due to adaptation to cold water temperatures (Chantai et al., 1996). It was also recently seen (C. Thongraung and M. Pazos, 2003, Personal Comunication), that Hb from a related cold-water living species, cod, showed very high pro-oxidative activity. Among the other species tested, flounder lives at more shallow, and thus, warmer waters. Mackerel and menhaden are "surface swimmers", and thus, are also subjected to more tempered waters compared to pollock. This hypothesis is further supported by recent unpublished findings showing that Hb´s from tropical fish have a low pro-oxidative activity compared to Atlantic species. It is supported by the finding that both deoxygenation and the ability to oxidize washed cod mince lipids during ice storage ranked trout, chicken and beef Hb´s as follows: trout >> chicken > beef (Richards *et al*., 2002). Finally, it cannot be excluded that different levels of Hb-effectors in the erythrocytes from the four species contributed to our findings. Organic phosphates represent the main mechanism of intraspecific Hb-adaption (Weber, 2000), and different levels/types of phosphates could thus be an important explanation to the different deoxygenation/autoxidation patterns seen for the four hemoglobin species at a given pH.

Conclusion

From this paper, it is obvious that acidification within the span seen in *post mortem* fish muscle (pH 7 to 6) accelerate Hb-deoxygenation and Hb-autoxidation. For flounder, menhaden and mackerel, these reactions of Hb correlated with stronger capacity to oxidize washed cod mince lipids at pH 6 than at pH 7. Pollock Hb was the most pro-oxidative at both pH 7 and pH 6, and was unaffected by the pH-change. It also had the highest deoxygenation/autoxidation rates at both pH´s. The unexpected differences between the studied species at pH 7 show that pelagic migratory fish do not necessarily have more pro-oxidative Hb because of their need for oxygen to carry out long-distance migratory swimming. Other factors, such as e.g. the water temperature (depth of living) and burst swimming seem more important based on the extremely pro-oxidative properties of pollock Hb.

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L 52. EFFECT OF HEMOGLOBIN TYPE ON LIPID OXIDATION PROCESSES IN FISH MUSCLE

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Abstract

The objective of these studies was to examine the relative ability of different fish hemoglobins to i) undergo hemoglobin autoxidation, ii) react with hydrogen peroxide and iii) promote lipid oxidation. Terrestrial hemoglobins were also examined for comparative purposes.

The soluble contents of erythrocytes were passed through a DEAE cellulose column to purify the hemoglobins. Hemoglobin oxygenation, autoxidation, and reaction with hydrogen peroxide were determined spectrophotometrically. Washed minced cod muscle was used as the lipid substrate. Lipid oxidation indicators measured during storage at 2°C were lipid peroxides and thiobarbituric acid reactive substances (TBARS).

At pH 6.3, deoxyHb content was highest in trout Hb, intermediate in chicken Hb and lowest in beef Hb. Autoxidation rate, reaction with hydrogen peroxide, and lipid oxidation rate were also highest in trout, intermediated in chicken and lowest in beef Hb. Anodic trout Hb (high deoxyHb content) was a better catalyst of lipid oxidation than cathodic trout Hb (low deoxyHb content). Autoxidation and lipid oxidation rate due to perch Hb was more rapid than trout Hb despite the lower deoxyHb content in perch Hb at pH 6.3.

These studies indicate that factors in addition to deoxyHb content affect the pro-oxidative nature of different hemoglobins. Role of heme crevice volume and differing amino acids in the heme crevice are discussed. These studies suggest that hemoglobin-mediated lipid oxidation in fish muscles depends not only on heme protein concentration (e.g. light vs. dark-fleshed fish) but also the type of endogenous hemoglobin.

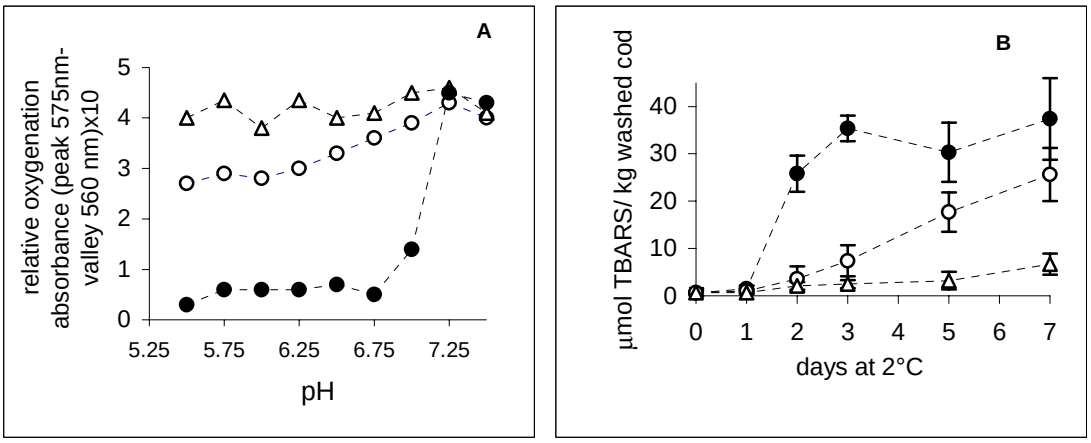

Figure 1. (A) Oxygenation of trout (•), chicken (o), and beef (Δ) hemoglobins at various pH values. (B) Lipid oxidation induced by each hemoglobin in washed cod muscle during 2°C storage at pH 6.3 as expressed by TBARS (Richards et al., 2002a).

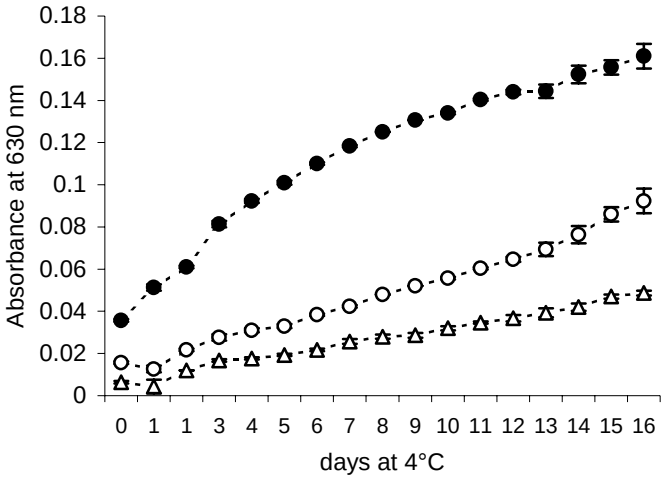

Figure 2. Autoxidation of trout (•), chicken (o), and beef (Δ) hemoglobins (pH 6.3).
Superoxide dismutase and catalase were added to prevent hypervalent forms of heme proteins from forming during incubations.

Table 1. Rate of metHb formation in solutions containing
20 μM hemoglobin, 50 μM H_2O_2 and 100 μM sodium azide.

Specie	% metHb formed/min
Trout	5.5 ± 0.2
Chicken	3.2 ± 0.1
Beef	1.5 ± 0.6

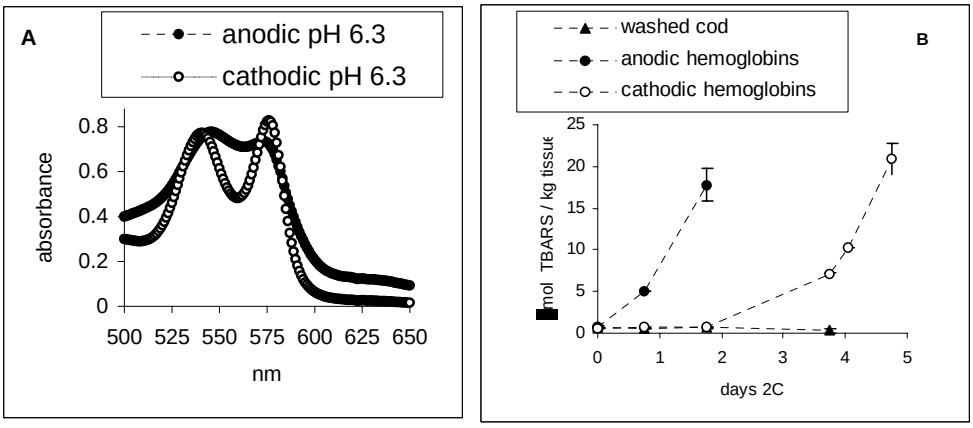

Figure 3. (A) Spectra of anodic and cathodic hemoglobins at pH 6.3. A deeper valley between peaks at 580 and 540 is indicative of lower deoxyHb content. (B) Lipid oxidation induced by anodic and cathodic hemoglobins at pH 6.3 as expressed by TBARS (Richards et al., 2002b).

Table 2. metHb formation in aqueous solutions (pH 6.3) and lipid peroxide formation in washed cod muscle (pH 6.3) due to trout or perch hemoglobins.

days at 4°C	% metHb		hours at 4°C	µmol lipid peroxides /kg washed cod	
	Perch	Trout		Perch	Trout
0	8.9 ± 0.6	2.3 ± 1.0	0	131 ± 9	3.6 ± 3.3
1	46 ± 3.9	15 ± 1.7	12	414 ± 78	6.7 ± 0.5
2	73 ± 6.1	26 ± 2.5	20	454 ± 98	54 ± 35
3	87 ± 7.7	36 ± 3.1	28	470 ± 88	156 ± 50
4	96 ± 4.4	43 ± 3.3	36	453 ± 39	223 ± 36
5		52 ± 4.4	44	397 ± 26	292 ± 19
6		57 ± 5.0	---	--	--

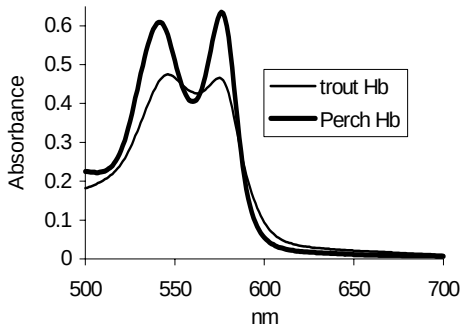

Figure 4. Spectra of perch and trout hemoglobins at pH 6.3. A deeper valley between peaks at 580 and 540 is indicative of lower deoxyHb content.

References

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L 53. SELECTIVE INCORPORATION OF A LIPID-SOLUBLE ANTIOXIDANT INTO THE MEMBRANE FRACTION OF FISH MUSCLE

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Abstract

The lipids of the cellular membranes of muscle foods are generally considered to be more susceptible to oxidation than the triacylglcerols (TAG). The objective of this research was to investigate the incorporation of exogenously added δ-tocopherol into the membrane lipid and TAG fractions of a model system of minced cod muscle and vegetable oil and to determine whether the antioxidant could be directed selectively into the membranes. The cellular membranes and the TAG were separated physically after the δ-tocopherol was added to the cod muscle in ethanol or canola oil. Also the order of addition of the tocopherol and the TAG was interchanged. The amount of tocopherol in the membrane and TAG fractions was determined using HPLC. When the lipid-soluble antioxidant δ-tocopherol was mixed with cod muscle tissue and canola oil, the ratio of the antioxidant in the membrane fraction to that in the oil depended on the order of addition of the oil and the antioxidant and the nature of the carrier used for the tocopherol. The ratio of the antioxidant in the membrane lipids to that of the oil was up to five times greater when ethanolic tocopherol was added before the oil rather than after. There was some improvement in the accumulation of the antioxidant in the membrane with ethanol as the carrier vs. the oil when oil was present before the antioxidant. When a solid fat was used, little tocopherol went into it. The results provide an understanding of how a lipid-soluble antioxidant is distributed between the neutral lipids and the membrane phospholipids when added to a complex food system. It also provides a way for substantially increasing the amount of antioxidant in the membranes by varying the order of addition of oil and antioxidant in multi-ingredient foods.

Introduction

Muscle tissue is a heterogeneous system with a continuous aqueous phase. Within this aqueous phase are located various proteinaceous structures. In addition, there are several membrane networks and membranous organelles. These membrane systems are comprised of polar lipids that exist as a bilayer with the polar groups directed towards the aqueous phase and the hydrophobic chains in the interior. Various proteins and other molecules are found in these membranes. There also exist oil droplets both inter- and intracellularly.

A major problem related to the use of many muscle tissue foods is the susceptibility of the tissue to oxidation of its lipid components. This is a special problem with fish from cold waters (most of the ocean) because their pro-oxidant systems are active at low temperatures and their fatty acids have a high degree of unsaturation. This oxidation problem is exacerbated when the muscle tissue is minced since this exposes lipid substrates to pro-oxidants and additionally often incorporates oxygen from the air into the mixture. It is generally recognized that the phospholipids of the muscle membranes are the lipids most susceptible to lipid oxidation.

One of the approaches to minimize oxidative changes in the tissue lipids is to add exogenous lipid-soluble antioxidants. The addition of these antioxidants usually requires a carrier. This carrier may be hydrophobic (low dielectric constant) or a relatively polar solvent like propylene glycol (high dielectric constant). The amount of carrier added plus the lipid-soluble antioxidant (AO) make up a very small proportion of the total mass of the muscle food.

We set out to answer several questions. Where does a lipid-soluble AO go when it is added into a mass of muscle in a small volume? How does the polarity (dielectric constant) of the carrier affect the distribution of the AO amongst the various cellular components, particularly the cellular membranes and the oil (triacylglycerol) droplets? If a lipid-soluble AO is added in an oil, will it be able to enter through the polar groups of the membrane surface to mix with the hydrocarbon chains of the membrane lipids? When a lipid-soluble AO is added in a polar solvent, it would be expected that the polar solvent would become diluted exposing the AO to water leading to agglomeration or the formation of micelles, and thus not reaching either of the main lipid fractions of the membrane or the oil droplets. Another question addressed was whether there were circumstances which would favor or disfavor the incorporation of the lipid-soluble AO into either of the two major lipid fractions.

Materials and methods

Cod fish was chosen as the muscle tissue for evaluation. This was based on its having a low lipid content almost entirely located in membranes. To simulate a fatty fish, canola oil was mixed with minced cod muscle. This provided a liquid triacylglycerol that was reasonably stable to oxidation and thus unlikely to destroy the δ-tocopherol added as the antioxidant. For the most part, the antioxidant was mixed into the muscle tissue either in

canola oil or dissolved in ethanol. The comparison of TOH uptake by the two lipid fractions throughout this report is based on concentrations of the TOH in lipids and not on absolute amounts.

To determine the distribution of the δ-tocopherol, the two major lipid fractions had first to be isolated physically from the mixture. Oil was obtained by applying pressure to the mixture via centrifugation. The mixture of minced muscle and oil was packed into centrifuge tubes and centrifuged with sufficient force to obtain the oil. Membranes were isolated from the muscle tissue by homogenization in neutral phosphate buffer solutions followed by differential centrifugation.

Results and Discussion

When δ-tocopherol (TOH) in ethanol was added to lean cod muscle, approximately 30% of the TOH was found in the membrane fraction. Since there is no oil in the minced cod muscle, it was presumed that the rest of the antioxidant formed insoluble aggregates in the aqueous phase or was bound to proteins. If oil were added to this system, the oil incorporated some antioxidant. At the same time, there was a decrease in the amount of TOH in both the membrane fraction and the aqueous phase, indicating that the oil obtained TOH from both of these fractions.

A simulated fatty fish was obtained by mixing canola oil with minced cod. TOH was added to this mixture in either ethanol or canola oil. A greater amount of antioxidant was incorporated into the membrane fraction when it was added in ethanol compared to when it was added in oil. Presumably the greater polarity of the ethanol compared to the oil allowed a better interaction of the TOH with the polar surface of the membranes. Ethanol itself is capable of being taken up by biological membranes.

From these results it appeared that there was a competition between the membrane lipids and oil for the available tocopherol which was modified by the dielectric constant of the carrier. The next set of experiments reversed the order of addition of oil and ethanolic tocopherol. When the ethanolic tocopherol was added to the minced cod muscle before the oil was mixed in, the concentration of tocopherol in the membrane was much higher than when the oil was present in the minced cod before the addition of the ethanolic TOH. This relative increase in uptake of TOH from the ethanolic system into its membrane lipids was markedly affected by the total amount of lipid present. This is undoubtedly due to the fact that the higher amount of tocopherol added in the presence of a higher oil content (to keep the concentration of AO in the total lipids constant), but added before the oil, favored the uptake of tocopherol into the membranes. When a solid triacylglycerol (beef fat) was substituted for the liquid canola oil, the concentration of TOH in the membrane was unaffected whether the solid fat was added before or after the ethanolic antioxidant solution. This implies that to a large extent lipid-soluble AO can be prevented from being taken up into TAG that is solid.

A study was also done to evaluate the uptake of TOH as a function of varying the dielectric constant between 18-32. Both the amount of TOH taken up into the membrane fraction and the amount that was not taken up into either lipid fraction was dependent on the dielectric constant. Although the ratio of the amount of TOH taken up by the membrane to that in the oil remained high at a high dielectric constant, the total amount of TOH taken up by the two lipid fractions decreased sharply in propylene glycol (dielectric constant of about 32).

Conclusions

The concentration of δ-tocopherol in the membrane fraction of minced muscle plus oil depended on:

1. The order of addition of TOH and oil to the muscle. The addition of tocopherol to the cod muscle before the addition of oil resulted in a higher membrane tocopherol concentration irrespective of the antioxidant carrier solvent used.

2. The dielectric constant of the antioxidant carrier had an important effect both on the distribution of the TOH between the two lipid fractions and the total amount taken up by both lipid fractions.

3. The physical state of the triacylglycerol fraction was important. A solid fat appeared not to readily take up the antioxidant.

4. Some of the TOH remained in the aqueous phase either as insoluble aggregates or associated with other components such as proteins. Adding oil to this mixture incorporated some of this aqueous phase TOH into the oil.

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L 54. PROTEIN SOLUBILITY OF HERRING PROTEINS AS AFFECTED BY FREEZING AND FROZEN STORAGE

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Abstract

Herring (*Clupea harengus*) is under-utilised for human consumption. One of the problems with the development of new and improved food products from herring is the variability in the chemical composition of the raw material and its seasonality. Food companies that rely on herring as an ingredient in their products have to know the quality and stability of their raw materials in order to produce final products with consistent quality all year round. The aim of this work was to evaluate the effect of freezing and frozen storage on the protein solubility of herring proteins. The long-term objective was to observe if it's possible to ensure that the raw material is stable from one herring season to the next for protein isolation. The project was funded by the Icelandic Centre for Research and the Nordic Industrial Fund. Fresh herring caught in two different seasons in January and September 2001 was used. It was filleted, skinned, packed in blocks and frozen in a plate freezer at an on-shore fishery plant. Samples were taken before freezing and after different time of frozen storage at -24°C. The shelf life of herring fillets was evaluated by chemical analysis (TBA-value, peroxide), viscosity and the protein solubility measurements at different pH.

Materials and Methods

Raw material. The herring used was caught South East of Iceland. In trial one in January 2001 the herring was about 12 hours post catch when landed. After landing the herring was size graded, beheaded, filleted and skinned by machines. Approximately 60 pcs. of fillets were packed in bags of 1 kg. Each bag was frozen (plate freezer) and sent two days later to IFL, Reykjavik. At arrival at IFL the fillets were put in frozen storage at –24°C and kept there until sampled. The herring used in trial two was also caught to the South East of Iceland but in September. The herring was landed after about 24 hours post catch. It got the same handling as the herring in January, size graded, beheaded, filleted and skinned by machines. After skinning the herring fillets were sent fresh, iced to IFL, Reykjavík. The fillets were at the institute 48 hours post catch. After arrival at IFL the fillets were packed in 1 kg. bags and frozen at –24°C. Parts of the fillets were kept on ice until analysed. The time from catch until landing and freezing for the herring caught in September was therefore longer than in January (Table 1).

TABLE 1. RAW MATERIAL IN TRIAL 1 AND TRIAL 2.

Trial	Fishing area	Time	Landing	Freezing
1	South East	January	12 h	12 h
2	South East	September	24 h	48 h

In trial one sampling took place after 1, 3, 6, 9 and 12 months of frozen storage at -24°C. In trial two samples were taken before freezing and after 7 days, 1, 3 and 6 months of frozen storage at –24°C.

Protein content was measured with a version of the original Kjeldahl method in a Kjeltec Auto sampler 1035/30 system. **Water content** in muscle was measured as weight loss after 4 hours at 103°C ±1°C. **Fat content** was determined by the method of Soxhlet (AOCS, 1990). **Salt content** was determined by a modified method of Volhard (AOAC, 1990). **TBA** (thiobarbituric acid) was measured by slightly modified steam distillation method (Tarladgis et al. 1960). Results were expressed in terms of mg malonaldehyde (MA)/kg tissue. **Peroxide value** was determined using ferric thiocyanate method (AOCS 1990). The peroxide value was calculated as meq/kg lipid. **Soluble Protein** was measured by the Biuret method (Layne, 1957; Torten and Whitaker, 1964). The **viscosity** was measured after proteins solubilization. The homogenates were placed into a 300 ml Pyrex beaker on ice. Viscosity was measured at 4-6°C using Bohlin visco 88 BV with # C 30 spindle and program number 6 at the highest speed 8 (1000 rpm).

Results and Discussion

Chemical compositions of the samples used in the two trials differed mainly in fat composition with the fillets in September being higher than in January. This is in line with previously reported results (Einarsson, 1998).

Table 2. Chemical composition of herring fillets used in the different trials.

	Protein [%]	Fat [%]	Water [%]	Salt NaCl [%]	Ash [%]
Trial 1 (Jan)	17,8 ± 0,1	10,7 ± 0,2	70,2 ± 0,2	0,4 ± 0,1	1,5 ± 0,0
Trial 2 (Sept)	18,1 ± 0,2	12,4 ± 0,2	68,0 ± 0,1	0,2 ± 0,1	1,4 ± 0,0

TBA values increase during frozen storage (Figure 1) and are higher in samples from trial two than one. Two possible reasons may explain this. The herring caught in September had higher fat content than in January. The time before freezing was longer for the September fillets than the January fillets. The start quality of raw material is very important for stability of frozen food products.

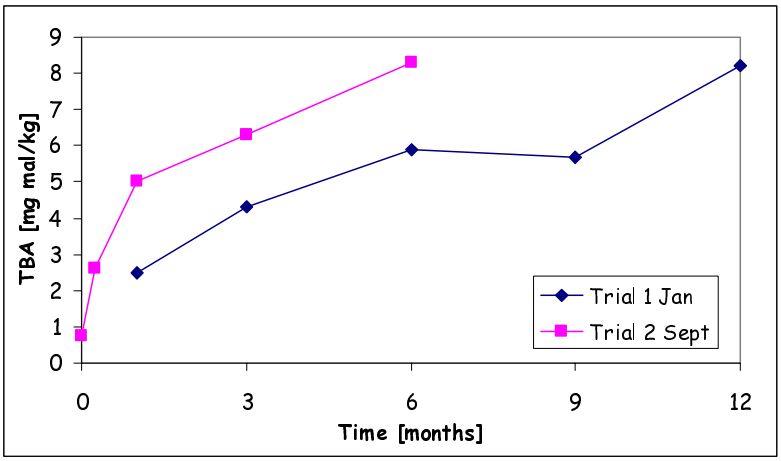

Figure 1. Changes in TBA-value in herring fillets stored at -24°C for up to one year. Trial 1 – herring caught in January, Trial 2 – herring caught in September.

Similar results are obtained for Peroxide value (Figure 2).

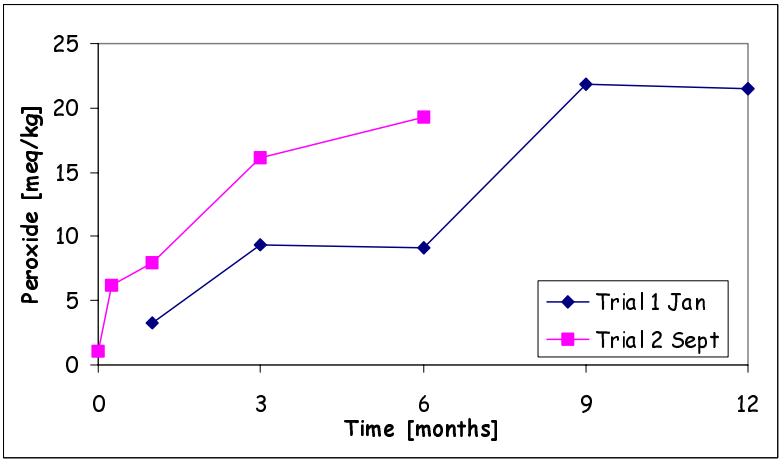

Figure 2. Changes in Peroxide-value in herring fillets stored at -24°C for up to one year. Trial 1 – herring caught in January, Trial 2 – herring caught in September.

Protein solubility decreased in both trials with frozen storage. After six months the solubility was around 5 % lower than in the start for trial 2 measured at pH 2.7 where the protein solubility is highest. For trial 1 the solubility lowers even more. After 12 months frozen storage the solubility increases again. Viscosity measured at pH 2.7 increases with frozen storage of the herring fillets. Freezing affects viscosity, after 7 days it had already increased and increased even more with longer frozen storage.

Conclusions

Frozen storage of herring fillets affected both protein solubility and viscosity of homogenate of herring at pH 2.7 where the solubility is highest.

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L 55. THE ROLE OF WATER STRUCTURING IN MUSCLE PROTEIN CRYOPROTECTION

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Abstract

Adequate cryoprotection of seafood meats, minces, or surimi likely depends upon proper structuring of water in the system. Water structuring in muscle also contributes to decreased ice formation and the associated damage inflicted by mechanical damage and freeze concentration of solutes during frozen storage.

Currently two separate mechanisms have been proposed to explain the cryoprotective effects of simple vs. polymeric carbohydrates added to muscle, or muscle purees like mince or surimi.

The presence of low molecular weight compounds such as sugars, glycerol, and polyols (sorbitol, mannitol) in a protein sol is known to favor the more folded, and therefore less reactive, form of the protein. The low molecular weight solutes have several effects:

(1) The carbohydrates are excluded from the protein surface (Timasheff and others, 1998) and structure water in the bulk solvent by enhancing hydrogen bonding (as revealed by an increased surface tension of the bulk water; McDonald and Lanier, 1996). This leads to greater viscosity in the solvent, particularly as unfreezable water is concentrated due to ice formation during freezing. The rates of diffusion-limited reactions, such as protein aggregation, are therefore lowered, particularly during frozen storage.
(2) Protein aggregation is further constrained by enhanced intramolecular hydrophobic bonding of proteins in the presence of these carbohydrates (Back and others, 1979) and folding of the protein resulting from the extremely unfavorable exposure of the peptide backbone to the carbohydrate solute (the opposite effect from that of denaturants such as urea; Liu and Bolen, 1995). These counteract the weakening of intramolecular hydrophobic bonds that normally accompanies cooling, caused by greater structuring of water as it approaches the freezing point.

Long chain carbohydrates, such as maltodextrin or polydextrose, are thought to stabilize dispersed proteins in frozen storage by limiting mobility of the water and thereby increasing the glass transition temperature (Tg') (Levine and Slade, 1988; Carvajal and Lanier, 1999). Under these conditions, the system forms less ice and more glass, resulting in less concentration of salts and dramatic decrease in the rate of diffusion limited reactions.

Surprisingly, however, the Tg' of seafood meats alone is already quite high, with apparent values for mackerel and cod being reported to be between –11 to –13 °C (Brake and Fennema, 1999; Carvajal, Amato and Lanier, recent observation). In contrast, there is a considerable body of evidence that most hydrated globular proteins undergo a glass-like transition at -93 to –53°C (Gregory 1995). This indicates that the long-chain polymeric nature of proteins cannot be responsible for the high Tg' of meats, as proposed by Brake and Fennema (1999).

Ling (1992) explained the limited mobility of water in muscle as the result of dipole layering of water (a different form of water structuring) by extended proteins. The extended proteins layer the intracellular water molecules mainly through their interactions with the exposed peptide backbone.

Solutions of fully extended proteins, such as gelatin or antifreeze glycoproteins, have this same physico-chemical effect on the water domain immediate to the protein surface (Ling, 1992). Gelatin and collagen fractions isolated from mackerel also yielded apparent Tg' values as high as –7°C (Brake and Fennema, 1999).

During rapid cooling of meats, the glass transition temperature, which denotes a phase change to an arrested metastable state of the water, is likely attained at a higher temperature because the water is already of greatly restricted mobility. The more fully extended proteins within intact muscle cells apparently induce the glass state of water more easily than do globular or non-extended fibrillar proteins. Hydrated globular proteins without added cryostabilizers show glass transition temperatures closer to that of bulk water (about -113 °C) indicating that water at the interface with these proteins associates weakly.

A secondary cause of damage to intact postrigor seafood meats during freezing results from dehydration of cells and structural damage caused by extracellular ice formation at freezing rates conventionally used. As ice forms, concentration of extracellular solutes due to ice formation raises the osmotic pressure outside the cell. Water movement out of cells facilitated by this osmotic pressure results in increasing amounts of extracellular ice formation, leading to distortion of the meat structure, and drip loss upon thawing. If the freezing rate is sufficiently fast there is insufficient time for water to move out of the cell and some intracellular ice formation can occur. An even more rapid freezing rate results in vitrification (glass formation) of the cellular water rather than ice formation. (Wolf and Bryant, 1999)

Based on Ling's (1992) view of muscle cells as consisting of extended proteins causing dipole layering/structuring of the intracellular water, ice formation within muscle cells would not be favored. Rather, supercooling and vitrification of intracellular water would predominate, even at freezing rates that are only rapid enough to avoid extracellular ice formation. Mazur (1970) observed that "…cells generally remain unfrozen … to

–10 or –15 °C, even when ice is present in the external medium…". This dipole layered water structure of intracellular water is consistent with our recent measurements indicating restricted diffusivity of proteins within muscle cells as compared to in bulk solution (Carvajal and Lanier, 2002).

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L 56. OXIDATION OF COD LIVER OIL - USE OF NATURALLY OCCURRING ANTI-OXIDANTS

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Abstract

The use of synthetic antioxidants has been shown to delay oxidative destruction of oil-containing systems, but little is known of the effectiveness of naturally occurring antioxidants in fish-oil systems. The objectives of this study were firstly to select suitably effective anti-oxidants from a range of naturally occurring components and secondly to determine the stability of cod liver oil containing anti-oxidants at different concentrations and at different temperature conditions. The extracted oil was divided into ten 150 ml batches and mixed with eight separate natural antioxidants (0.5 % w/w of tea catechin, α-tocopherol natural and synthetic, black or white clove oil, mustard, rosemary or carvacol), a synthetic antioxidant, BHT (0.05 % w/w) and a control. All samples were stored at 30°C in darkness for 16 days. Peroxide, anisidine (a measure of primary and secondary oxidation products in oil, respectively) and totox (peroxide and anisidine combined) values were measured every second day over a 16 day period. Oil samples containing tea catechins and rosemary had lower levels of oxidation than those containing the other anti-oxidants. The stability of extracted cod liver oil was then measured on fresh samples containing 0.1, 0.25 or 0.5% w/w of tea catechin or rosemary. The analysis was performed under accelerated oxidation conditions at 60 or 80 °C using the Oil Stability Index method. During these conditions, rosemary added at 0.5% w/w was shown to be the most effective anti oxidative agent, followed by tea catechin at 0.5% and rosemary at 0.25%.

These results are part of the EU-project "Utilisation and stabilisation of by-products from cod species" (QLK-CT2000-01017)

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L 57. DETECTION OF BIOCHEMICAL DAMAGES DURING FROZEN STORAGE OF HORSE MACKEREL SURIMI.

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Abstract

During processing and storage, the quality of fish and fish products may decline due to a variety of causes among them lipid and protein alterations. Indeed, lipid oxidation increases during manufacturing of horse mackerel surimi but processing conditions can be designed to minimize its development and to produce a surimi of satisfying quality. To maintain this initial quality, a further control of the development of lipid oxidation in the product through good storage conditions is also required. Frozen storage allows greatly increasing the shelf-life of the products. However, biochemical reactions like lipid hydrolysis, lipid oxidation and protein denaturation, can occur during frozen storage of fish products resulting in texture and flavour modifications.

The aim of this study was to monitor changes in biochemical properties of horse mackerel surimi during storage at –20°C, under or without vacuum. Primary (hydroperoxides) and secondary (thiobarbituric acid reactive substances = TBARS) lipid oxidation products and protein alterations (tryptophanyl residues (Trp) fluorescence) were measured after four, eight and twelve months of storage.

The horse mackerel surimi contained 1.1 g lipids per 100g of wet product. The lipids were highly unsaturated as total polyunsaturated fatty acids accounted for 33 % total fatty acids. Initial contents of hydroperoxides and TBARS in surimi were respectively 0.29 mmoles equivalents cumen hydroperoxides per Kg and 0.46 mg equivalents malonaldehyde per Kg. Contents in these products of lipid oxidation and Trp fluorescence intensity decreased during frozen storage. The decrease in TBARS was the more pronounced during storage under vacuum and Trp fluorescence intensity was the lowest after twelve months of storage without vacuum.

Owing to the presence of oxygen when surimi was stored without vacuum, one may suspect that lipid oxidation is favoured as compared to the product stored under vacuum. As the measured lipid oxidation products are a balance between produced and decomposed, or further reacted products, the observed decreases in lipid oxidation products and Trp fluorescence intensity probably result from decomposition and interactions of lipid oxidation products with proteins.

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L 58. PROTEINS FROM SARDINE AND BLUE WHITING RECOVERED BY NEW EXTRACTION TECHNIQUES: SOLUBILITY AND GELATION PROPERTIES

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Abstract

Formation of cross-linking induced by added microbial transglutaminase (TGase) (0 – 8.0 units TGase/g actomyosin) was investigated using SDS-PAGE. The intensity of the band corresponding to the myosin heavy chain did not show a clear decrease with increasing amounts of TGA until 1.0 unitTGase/g actomyosin added. Preliminary studies on the effect of TGA (0 – 20 mg TGase/g surimi) on the gelation properties of blue whiting surimi were developed. Changes in gel strength, hardness, cohesiveness and elasticity were measured. TGA affected the texture of the gels, being the highest values of gel strength and hardness reached at 0.5 and 1.0 units TGA/g, respectively. Elasticity showed a maximum value at 8 mg TGase/g whereas cohesiveness was independent of the level of TGase.

New techniques of fish protein recovery involving either acid or alkaline extraction have been recently patented. Protein solubility curves of sardine (*Sardina pilchardus*) and blue whiting (*Micromesistius poutassou*) were determined for selection of optimum pH values to be used in the extraction and precipitation processes. Yield of acidic and alkaline recovery of sardine and blue whiting protein was determined. Solubility curves were similar in both species. Lowest values occurred between pH 5.0 and 6.0. A sharp increase occurred at acidic pH between 4.0 and 2.5 and at alkaline pH between 9.5 and 11.0. Considerably higher yields were achieved in both species using the new techniques for protein recovery.

Materials and Methods

Sardine (*Sardina pilchardus*) and blue whiting (*Micromesistius poutassou*) caught off in the Portuguese coast were used in these trials. The preparation of blue whiting gels with different levels of TGase as well as texture measurements (gel strength, hardness, elasticity, and cohesiveness) and folding test were done as referred by Mendes *et al.* (1998).

Actomyosin was extracted from blue whiting muscle according to the method described by Noguchi and Matsumoto (1970).

Actomyosin (AM) extracts were incubated at 40 ºC with several concentrations of transglutaminase (0, 0.1, 0.3, 0.5, 0.7, 1.0, 3.0, 6.0, and 8.0 units /g of AM). To the extracts (100 µl) containing 1.5 mg of AM/ml the corresponding levels of TGase were added. After 1 hour incubation, the reaction was stopped with the addition of 0.4 ml Tris-HCl buffer (2 % SDS, 5 % 2-mercapto ethanol, 62.5 mM Tris-HCl, pH 6.8) and heating in a water bath (95 ºC) for 3 min. The samples were analysed by SDS-PAGE using 7.5 % polyacrylamide and silver staining.

The solubility of sardine and blue whiting myofibrillar proteins as a function of pH was studied following the methodology described by Stefansson and Hultin (1994). Solubility of blue whiting myofibrillar proteins was also measured in tap water (60 ppm $CaCO_3$) t different pH values. Protein was determined assayed according to the method of Lowry *et al*. (1951).

Application of transglutaminase

Transglutaminase (TGase) has been considered to be involved in the polymerisation of the myosin heavy chain during low-temperature setting. This enzyme is present in fish muscle but its level can be reduced due to processing. This fact has led to the addition of TGase to surimi to improve the gel formation.

The gel-forming ability of blue whiting surimi increased with the addition of commercial TGase. Gel strength reached a maximum when 0.5 units/g surimi of TGase was added (Fig. 1). The gel strength of the sample with 0.5 units / g surimi was 55 N.mm, which was about 5-fold of the control. Higher levels than 0.5 units /g of added enzyme led to a decrease in gel strength, which leveled off at around 40 N.mm. The occurrence of a maximum in gel strength was also observed in other surimi species (Sakamoto *et al.*, 1995, Tsai *et al.*, 1996, Jiang *et al.*, 2000).

Both hardness and elasticity increased with added TGase (Fig. 2). The results of the measurements presented are maximum values at 1.0 and 0.8 units /g surimi for hardness and elasticity, respectively. The increase recorded in the maximum value for hardness was about 2-fold of the control. The cohesiveness of the gels did not seem to be affected by the addition of TGase. All samples with added TGase showed the highest score (5) for the folding test whereas the control presented a lower value (4). These results indicate that TGase improved the folding capacity of the gels.

Changes in SDS-PAGE profiles of blue whiting actomyosin incubated with different levels of TGase were studied (Fig. 3). The intensity of the band corresponding to myosin heavy chain (MHC) did not show a clear gradual decrease with increasing amounts of TGase. The MHC of blue whiting samples with added TGase until 1.0 units /g AM did present important changes. However, for TGase concentrations above those levels the MHC almost disappears. Its disappearance and the lack of evidence of bands corresponding to new smaller molecular weight proteins support the occurrence of MHC cross-linking on account of the ε-(γ-glutamyl)lysine isopeptide bonds formed in the cross-linked MHC by TGase. Nevertheless, the bands corresponding to the components with higher molecular weight resultant from cross-linking did not evidence an increasing intensity with higher amounts of TGase. Technical analytical difficulties could be responsible for the unclear changes in the band of proteins involved in cross-linking.

New protein extraction techniques

In order to achieve a high proportion of available muscle protein it was recently developed a new method of protein recovery (Hultin and Kelleher, 2000 and 2001), which permits the use of muscle protein from different sources. This process for isolating the fish proteins involves the solubilisation of a disperse form of the fish tissue either in an acidic or in an alkaline aqueous solution. The protein rich aqueous solution is separated from solids and lipids and the protein is then precipitated and recovered by centrifugation.

The solubility in distilled water of sardine and blue whiting myofibrillar proteins was very low at pH 4.5-7.5 and increased as the pH is shifted to either side (Fig.4). The highest protein solubility was recorded at pH 2.5 and 11.5 – 12 and the isoelectric point of sardine proteins was around pH 5 – 5.5 for both species. The solubilisation of blue whiting myofibrillar proteins in tap water showed a 2-fold increase in protein extraction at extreme acidic pH. This increase of protein solubilisation at very low pH may be due to reinforcement of the net positive charge of protein as a result of the increased ion concentration of tap water. The pH range of low protein solubility was broader (5.5 – 9.5) than in the observed with distilled water. This broader pH range could result from the shielding effect of ions present in tap water on the negative charges of protein at these pH values. Extraction and protein recovery by this new process led to a significant improved yield. Lower recovery yields (53.6 % for acid-added recovery and 49.1 % for basic-added recovery) were obtained for blue whiting but they are de double of those achieved by the conventional process of surimi preparation (*ca.* 25 %). The yields obtained for sardine were 73 % and 77 % for the acid and basic protein recovery, respectively.

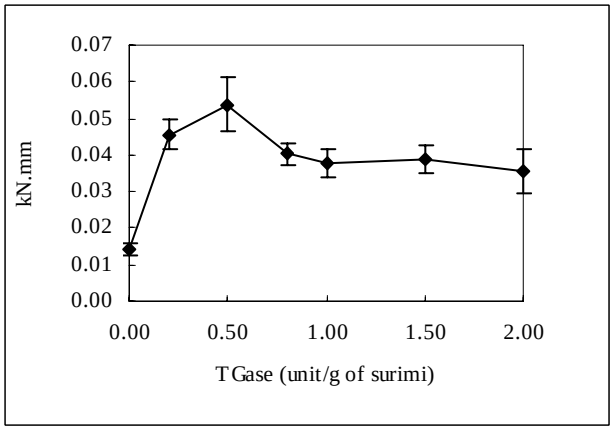

Fig. 1. Effect of TGase on the gel strength of blue whiting surimi. Gels set at 35 ºC for 60 min.

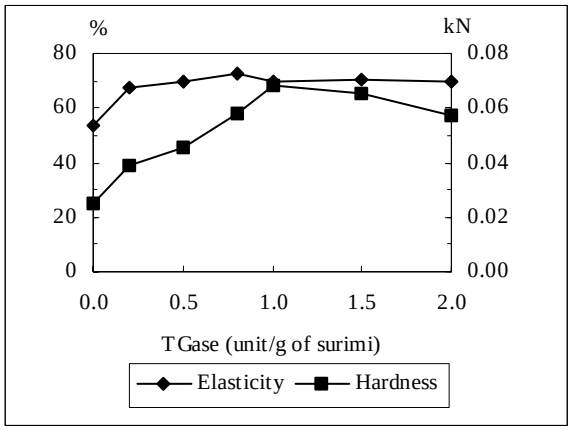

Fig. 2. Effect of TGase on the elasticity and hardnes of blue whiting surimi. Gels set at 35 ºC for 60 min.

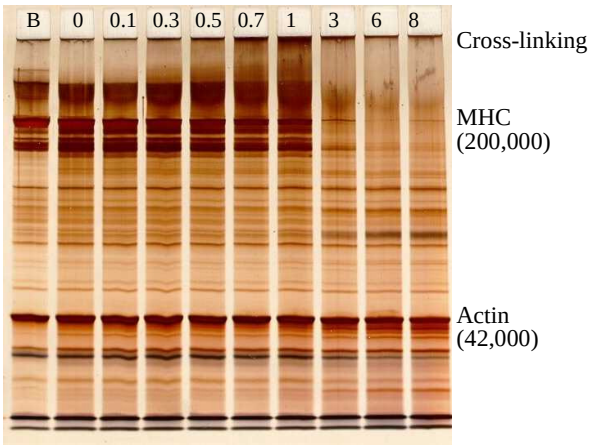

Fig. 3. Changes in SDS-PAGE profile of blue whiting actomyosin (AM). B – actomyosin extract without TGase and with various amounts of TGase.

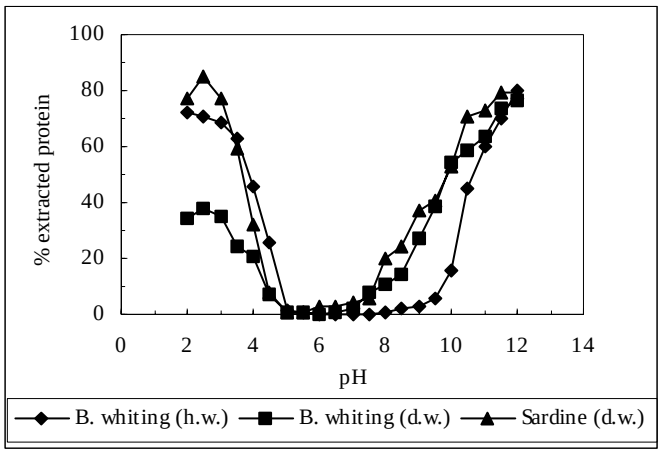

Fig. 4. Effect of pH on protein solubility of blue whiting and sardine myofibrillar protein fraction. d. w. – distilled water; h. w. – hard water.

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L 59. NEW WAYS TO GET A PROTEIN CONCENTRATE WITH GELLING PROPERTIES FROM SQUID MUSCLE

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Abstract

It makes sense to advance the technology of cephalopod muscle gelation for the manufacture of analogues and other new products, given that the muscle is white, has little flavour and practically no fat, and is in abundant supply throughout the world.

Published studies on gelation of squid muscle raise a number of difficulties, essentially relating to peculiarities of myofibrillar proteins and very high autolysis in its muscle.

To get round these problems we have devised a new procedure for processing frozen muscle of *Dosidicus gigas*, an underused species found in abundance off the West Coast of Mexico and Peru. The process is based on the solubilization and acid precipitation of much of the muscle protein. Different concentrations (0.05 M- 0.8 M) of NaCl and different proportions of solute-solvent have been assayed for better solubilization of muscle proteins. Different pH levels have been tested to optimize precipitation and preserve myosin. Filtration and centrifugation have been assayed for better collection of the precipitate. Different food-grade enzyme inhibitors have been tested to prevent myosin lysis and to obtain stronger gels. Following optimization of the processing conditions, the protein concentrate was stored at different pHs with conventional cryoprotectants and its gelling properties were measured throughout the course of frozen storage.

The best processing conditions were: Solubilization with 0.5 M NaCl, pH 7; solute/solvent ratio 1/6; pH for precipitation 4.5-5. None of the tested food grade enzyme inhibitors (potato powder, beef plasma protein) have been found to inhibit enzymatic activity, but if muscle is processed quickly at low temperature, there is minimal myosin lysis. Frozen protein is best stored at acid pH, although there is some loss of gelling properties in the course of frozen storage.

The maximum strength of gels made from this frozen muscle concentrate was around 300 g x cm (moisture 70-73 %). They presented a high degree of whiteness (whiting index: 80; L*: 79,5) and no impurities.

Results and Discussion

Solubilization of myofibrillar protein: Since squid muscle is solubilized in low ionic strength solutions (Tsuchilla et al, 1978), solubilization assays were performed on squid mantle with water and sodium chloride at concentrations ranging from 0.05 M to 0.8 M. Varying proportions of solute and solvent were also assayed. In this way it was determined that the most suitable solvent was 0.5M sodium chloride in a solute / solvent proportion of 1:7. In most cases there was no need to correct the pH, as it was close to neutral. Only in cases where the squid muscle was extremely acid (depending on size and season) was a buffer necessary. Homogenization should be performed in such a way as to avoid foaming, using either a vacuum cutter or a paddle stirrer.

Protein degradation: Squid muscle presents high protease activity (Konno and Fukazama, 1993; Ayensa et al, 1999; Konno et al, 2003). Protein degradation has been studied by electrophoresis in a chilled stored muscle extract dissolved in 0.5 M NaCl during 24 h. A gradual diminishing of myosin heavy chain (MHC) was observer until it disappear at 24 h. This diminishing of MHC happen at the same time than other bands at 132 KD (HMM) and 25 KD (LHM) increase. If the experiment is carried out at 25 ºC MHC disappear in 2 h. It has been seen that PMSF inhibited in some extent degradation of the myosin band; this is a sign of serin-protease activity.

Isoelectric protein precipitation: Protein precipitation was assayed at several pH values (Figure 1). Acid precipitation was selected, at a pH of around 4.5. Two methods were assayed to collect the precipitate, one by filtration and the other by centrifugation (3000 x g). The latter was selected as being more suited to subsequent industrial development.

Neutralization of protein precipitate: Various substances were assayed to neutralise the precipitate. The most suitable is a solution of 1M NaOH since at higher concentrations there is a risk of spontaneous gelification upon contact with the concentrate. Neutralisation must be performed at the time of gelification.

Frozen storage: The protein concentrate was mixed with a cocktail of classic cryoprotectants used in surimi (4% saccharose and 4 % sorbitol). Part was neutralized with 1 M NaOH and part was stored with acidic pH. Figure 3 shows a slow loss of gel strength over time in storage in the case of the concentrate stored with acidic pH. However, when the concentrate was neutralised before freezing, there was a practically total loss of gel strength after thawing.

Gelification: The protein precipitate had approximately 80 % moisture, a pH of 4.5 and around 2.5 % NaCl. Trials were run in which the protein was washed with distilled water to remove most of the NaCl and proteases then centrifuged again to collect the protein. The resulting protein had lower gel strength, and we therefore opted not to

wash. Thus, for gelification the dough formed by the protein, water and NaCl was neutralised with a solution of 1 M NaOH, 0.5 % sodium tripolyphosphate and 3 % potato starch and blended for 10 min in a vacuum cutter. The gelification profile is shown in figure 2. The profile gives no indication that this is a protein that undergoes setting, unlike muscle of other squids (Sano et al., 1989).
Further tests were performed on muscle with food grade inhibitors as bovine plasma, egg white and potato extract, following the recommendations of other authors but gel strengths were no greater than the control (Figure 4).

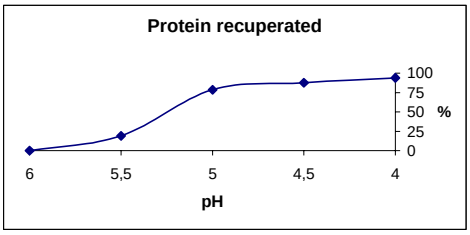

Figure 1. Percentage of recuperated protein by precipitation at different pH.

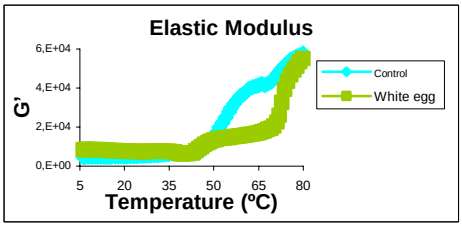

Figure 2. Changes in elastic modulus (G') during gelification by thermal treatment at 1ºC/min

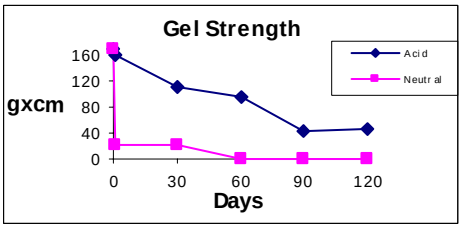

Figure 3. Gel Strength of concentrate with different pH ingredients along frozen storage.

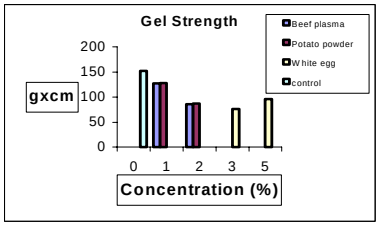

Figure 4. Gel strength with different

Conclusions

Using the method described above, we obtained a protein concentrate in an acidic medium from frozen muscle of *Dosidicus gigas*, with high gel forming capacity (around 300 g x cm with 70-73 % moisture), a high degree of whiteness and no impurities. When conventional cryoprotectants are added, gel properties are preserved for several months. To make a gel from this concentrate, the concentrate must be neutralised, ingredients added and the whole blended in a cutter. No salt addition is necessary since the concentrate contains an adequate proportion as from the first step of solubilization. The procedure has been patented in Spain (Careche et al. 2002).

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L 60. HYDROLYSIS OF COD (*GADUS MORHUA)* BY-PRODUCTS: INFLUENCE OF INITIAL INACTIVATION, AMOUNT OF ADDED WATER AND CENTRIFUGATION CONDITIONS

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Abstract

In recent years there has been an increasing demand for utilisation of fish by-products. Public awareness on environmental issues has also increased and companies are encouraged to develop new and modern processing methods, where pollution is reduced and energy is saved. Comparison of the composition of fish flesh and fish by-products shows that fish by-products has a high percentage of proteins and can be used to produce different valuable products.

The main aim of hydrolysis of by-products is to obtain the highest possible amount of valuable components with maximum quality and utility. Backbones and liver from farmed cod (*Gadus morhua*) were used for the experiment. The main objective of the present work was to evaluate how different factors such as initial inactivation of endogenous enzymes, amount of added water, centrifugation speed and time influence the yield of different fractions: pure oil, emulsions, fish protein hydrolysate (FPH) and sludge (non-soluble part). Differences in the quantitative and qualitative distribution of lipid classes were observed in the different fractions. Some tests were also performed in order to reduce the amount of the insoluble part fraction. Functional properties such as fat absorption, emulsifying properties and degree of hydrolysis were also tested.

Introduction

Many studies have been done on the evaluation of the conditions of the hydrolysis and functional properties of FPH based on whole fish, fish fillet or muscle. However only recently more works concerning utilisation of fish by-products has appeared in the scientific press: Jeon *et al*. in 2000; Gildberg in 2001, Gildberg *et al*. in 2002; Liaset et *al*. in 2002. However, neither the influence of added water nor the amount of added enzyme was studied, both which are of utmost economical interest in the hydrolysis process (Liaset *et al*., 2002).

One of the aims in the processing of fish by-products is to keep the costs of producing the hydrolysate as low as possible. All additional procedures during hydrolysis (introduction of bacteriostat, charcoal treatment, defatting with isopropanol) can increase the price of the final products. In case of by-products this is not desirable. Products should have as high quality as possible at the smallest possible price. One of the possible ways to achieve this is to reduce the amount of water in order to reduce expenses for heating and hydrolysing. Also, by reducing the amount of water expenses for evaporating and drying of hydrolysate can be reduced. However, during hydrolysis peptide linkages are being broken in the insoluble and soluble fraction. High concentrations of soluble peptides in the reaction mixture markedly reduce both the rate of hydrolysis, and also the yield of material released into solution (Mohr, 1977). This ratio should be optimised for the processes.

Furthermore, during heating to the temperature of hydrolysis the sarcoplasmic fraction can denature and precipitate. The denatured proteins are apparently highly resistant to enzymatic breakdown, consequently only a minor part of the sarcoplasmic fraction will be solubilised during subsequent enzyme hydrolysis (Mohr, 1979). These investigations suggest that the state of the substrate before hydrolysis may be of considerable significance. So, a “temperature programmed hydrolysis” should give measurably higher yields of soluble products than a conventional enzyme process. In addition, the amount of raw material being converted into soluble hydrolysate is a factor, that is especially important for industrial process (Mohr, 1977). However, even after 20 years not much work has been done on the evaluation and examination of the partly soluble part after hydrolysis. The applications and yield of the hydrolysis could be increased significantly by better description of all fractions after hydrolysis and finding application of partly soluble fractions.

The main objective of the present work was to evaluate how different factors such as initial inactivation of endogenous enzymes, amount of added water, centrifugation speed and time influence the yield and functional properties of different fractions. Furthermore the aim of this study was to evaluate how different factors influence the yield and functional properties of partly soluble fractions. With the aim of finding better utilisation of all protein parts generated during enzymatic hydrolysis.

Materials and Methods

Liver and backbones from farmed cod (*Gadus morhua*) was used in the experiment. Liver was homogenised by kitchen homogeniser and backbones minced twice in a HOBART mincer. The minced and frozen stored fractions were thawed and portions of backbones and liver were mixed at proportion 1:0.8. For the half of the mixture endogenous enzymes were inactivated in the microwave oven (5 minutes at 95 ^{o}C), while another part was placed in to the hydrolysis reactor directly after mixing with distilled water. These mixtures were warmed up in the water bath to a temperature of 55^{o}C. First part of experiment was performed by mixed raw material with an equal volume of distilled water: 1:1 (Kristinsson and Rasco, 2000). In the second part of experiment amount of added water was reduced till 75 % of the weight of initial mixture (1:0.75). The enzymatic hydrolysis was performed by adding 0.1% (by weight of raw material) of Alcalase 2.4 L (Novozymes A/S, Denmark) and kept for 60 minutes at 55ºC. After hydrolysis, the enzymes were inactivated in the microwave oven (5 minutes at 95^{o}C). The hydrolysed mixtures were filtered in order to separate the bone particles. The mixtures were centrifuged at 320×g, 1290×g and 2900×g speed for 10 and 30 minutes respectively in the "swing out" rotor. After centrifugation tubes with the samples were frozen and then all fractions were separated by cutting frozen content of the tubes and collected. All fractions: oil, emulsion, fish protein hydrolysate (FPH) and sludge were frozen. The FPH, emulsion and sludge fractions were freeze-dried.

Ash content was estimated by charring in a crucible at 600^{o}C until the ash had a white appearance (AOAC, 1990). The total N was determined by CHN-S/N elemental analyser and crude protein was estimated by multiplying total N by the factor 6.25. The extraction of total lipids from the samples was performed according to the method of Bligh and Dyer (1959). The degree of hydrolysis was evaluated as the proportion (%) of α-amino nitrogen with respect to the total N in the sample (Taylor, 1957). Amount of free amino acids was determined by reversed phase HPLC by pre-column fluorescence derivatization with *o*-phthaldialdehyde, using a NovaPak C18 cartridge (Waters, Milford, MA, USA) and the method of Lindroth and Mopper (1979) as modified by Flynn (1988). SDS-PAGE was performed on water-soluble proteins according to Laemmli (1970) and the gels were stained with Silver Staining. The ability of powders to bind oil was measured according to the method of Shahidi *et al.* (1995) with some modifications. Emulsification capacity was measured by mixing soybean oil with 5 % FPH and sludge solution in water, homogenising and centrifuging. Emulsification capacity was expressed as millilitres of emulsified oil per 1 g of FPH or sludge (Kinsella, 1976*).* Emulsion stability was expressed as the percentage of initial emulsion remaining after on day at room temperature and centrifugation (McClements, 1999). Lipid classes were determined by thin-layer chromatography (Rainuzzu *et al*. 1992). Statistical program Guidelines (CAMO ASA, Oslo, Norway) and Microsoft Excel were employed for data processing and statistical analysis. Significance level was determined at the 95% level.

Results

During hydrolysis, by-products were converted into yellow-brownish liquid mixtures with bone particles. After centrifugation, the FPH were clear yellow and sticky liquids, emulsions – whitish fatty mass, sludge – dust colour bottom fraction. The dried FPH powders had light yellow colour and a fishy odour. Emulsions were sandy grey coloured fatty mass. Sludge was brown- dusty coloured powders.

The most important factor for the yield of protein containing fractions was initial heating of the system rather than amount of added water. Samples with active endogenous enzymes give significantly higher yields of FPH and reduced amount of sludge. Centrifugation conditions influenced yields of oil containing fractions, while inactivation of endogenous enzymes and different amount of added water did not show significant differences. Higher speed and longer time of centrifugation increased the volume yield of oil and reduced the volume yield of emulsions.

The insoluble sludge contained a high amount of oil including high concentration of phospholipids. Initial heating increases amount of phospholipids in the sludge. Samples after inactivation of endogenous enzymes had less free amino acids, lower degree of hydrolysis and formed more stable emulsions. Amount of added water prior to hydrolysis influences the physiochemical and functional properties of FPH and sludge: FPH and sludge obtained with lower amount of added water had higher concentration of phospholipids and showed higher emulsifying capacity. Powders obtained with lower amount of added water had lower fat absorption ability.

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L 61. PROPERTIES OF HYDROLYSATES FROM PINK SALMON HEADS AND VISCERA

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Abstract

Five species of Pacific salmon are commercially harvested in Alaska (genus *Oncorhynchus)*, with pink salmon (*Oncorhynchus gorbuscha*) harvested in the highest volume. The major by-products of salmon processing are heads and viscera. The objective of this study was to make dried hydrolysates containing all protein and fat from both salmon heads and viscera and characterize their chemical and nutritional properties.

Approximately 1,000 kg of both fresh pink salmon heads and viscera were obtained from commercial plants and immediately processed in a pilot plant sized hydrolysis unit (ASTA Ltd.). Heads or viscera were continuously ground, mixed with a constant amount of papain and the hydrolyzed at 61-65 °C. The reaction was terminated by denaturing papain at approximately 85 °C and the hydrolysates concentrated in an evaporator to 40-45% solids. At a latter time hydrolysates were dried to powder using a Littleford dryer and then mixed with ethoxyquin to 150 ppm. Characterization of hydrolysates included, proximate analysis, amino acid profiles, mineral analysis, fat characterization, percent peptide bond cleavage with the OPA method (DH), protein solubility and size, pepsin digestibility and physical properties.

Raw heads and raw viscera had a composition of 75.1 and 79.7 % moisture, 15.8 and 16.2 % protein, 3.2 and 1.5 % ash and by subtraction 5.9 and 2.6 % fat, respectively. Composition of the final dried head and viscera hydrolysates was 6.6 and 7.4 % moisture, 59.6 and 74.6- % protein, 5.5 and 6.8 ash, and 29.8 and 12.8 % fat, respectively. DH values of samples were 12.0 to 14.5 and 16.3 to16.9 for dried hydrolyzed head and viscera products, respectively. Pepsin digestibility values for both raw and hydrolyzed heads and for viscera was over 94%. Calculated rat PER values for the dried hydrolysates from heads and viscera were greater than 2.6 and 2.8, respectively. All major proteins present in raw heads and viscera were reduced in size to small peptides, amino acids or other compounds in the corresponding hydrolysates.

In conclusion, dried hydrolysates made without removal of fat from both pink salmon heads and viscera had many desirable properties.

Introduction

In Alaska there are five species of Pacific salmon (genus *Oncorhynchus)* that are commercially harvested. Total reported Alaska salmon harvest in 2000 was 319,471 metric tons resulting in an estimated 86,257 metric tons (MT) of fish processing byproducts (Crapo and Bechtel, 2003). It was estimated that the major salmon processing byproduct components were 57,000 MT of heads and 28,000 MT of viscera. Pink salmon (*Oncorhynchus gorbuscha*) are harvested in the highest volume and in Alaska and the major fish processing byproducts are heads and viscera. There are numerous studies on making hydrolysates from whole fish and combined fish byproducts but little information on the properties of hydrolysates made from individual processing byproducts such as heads and viscera. Often when hydrolysates are produced the oil fraction is separated from the aqueous fraction before concentration. The objective of this study was to make dried hydrolysates that contain protein and fat from both salmon heads and viscera and characterize their chemical, nutritional and functional properties.

Materials and methods

Approximately 1,000 kg of both fresh pink salmon heads and fresh viscera were obtained on separate days from commercial plants and immediately processed in a pilot plant sized hydrolysis unit (ASTA Ltd.). Heads or viscera were continuously ground through a plate with 0.5 cm size holes, continuously mixed with papain (Corolase L 10, AB Enzymes) and heated in a tubular scrap surface heat exchanger to the hydrolysis temperature. The heated raw material and enzyme were continuously pumped through the reactor tubes where the hydrolysis continued at 61-65 °C for a constant time. The enzymatic reaction was terminated by denaturing the papain at approximately 85 C using live steam. Bone was continuously separated from hydrolysate using a shaking screen separator. Hydrolysates with the bone removed were concentrated in a scrape surface falling film evaporator to 40-45% solids. At a latter time hydrolysates were dried to powder using a Littleford dryer and then mixed with ethoxyquin to150 ppm. A series of samples of the ground raw head, and viscera materials and corresponding hydrolysates (from which the bone was removed but before concentration) were taken at timed intervals after

processing conditions had stabilized. Multiple samples of the final dried products were taken after the ethoxyquin had been added and the samples mixed thoroughly.

Moisture content of samples was determined by drying at 103 °C for 17 hr in triplicate. Ash content was determined in triplicate from dried samples placed in a muffle furnace at 550 °C for 6 hr. Nitrogen content was determined in triplicate using a Leco FB-2000 nitrogen analyzer. Protein content was calculated as percent nitrogen times 6.25. Dried samples for mineral analysis were ashed overnight in a 550 °C muffle furnace. Ashing residue was digested overnight in an aqueous solution containing 10% (v/v) hydrochloric acid and 10% (v/v) nitric acid. Digested solutions were analyzed for Ag, Ca, Cd, Cu, Fe, K, Mg, Mn, Na, Ni, P, Pb, Sr, and Zn by inductively coupled plasma optical emission spectroscopy on a Perkin Elmer Optima 3000 Radial ICPOES. Amino acid content was determined by digesting dried samples in 6 N HCl for 24 hr at 110 °C. Precolumn derivitization was used and amino acid content was determined with a HPLC system. The estimated rat protein efficiency ratio (PER) was calculated from the amino acid analysis determinations. Pepsin digestibility of samples was completed using the AOAC Official Methods. Degree of hydrolysis (DH) was determined using the OPA method. Color (L*, a*, and b*) of the hydrolysate powders was determined using a color meter. The SDS-PAGE gel electrophoreses were performed on samples of hydroysates before and after concentration. Percent protein solubility was determined by a modification of the protein dispersibility index. Functional properties of the hydrolysate powders were determined and included nitrogen solubility, fat absorption capacity, emulsifying capacity and emulsion stability. Where appropriate ANOVA procedure were used with Statistica release 6, series software with two factors and dependent variables. Post-hoc analysis used the Duncan test and the level of significance used was $p<0.05$.

Results

Dried hydrolysates were made from both pink salmon heads and viscera that contained all the lipid found in the raw material. Both dried hydrolysates powders had good flow characteristics. The composition of raw heads and raw viscera was 75.1and 79.7 % moisture, 15.8 and 16.2 % protein, and 3.2 and 1.5 % ash, respectively. Composition of the final dried head and viscera hydrolysates was 6.6 and 7.4 % moisture, 59.6 and 74.6 % protein, 5.5 and 6.8 ash, and 29.8 and 12.8 % fat, respectively. Degree of hydrolysis for the dried hydrolyzed values ranged 12.0 to 14.5 and 16.3 to16.9 for dried head and viscera products, respectively. Protein digestibility was determined using a pepsin digestibility assay. As expected, the pepsin digestibility assay values for raw and hydrolyzed heads and viscera were high at over 94%. Amino acid analysis of samples indicated a high lysine content in both hydrolyzed head and viscera products. The amino acid contents of ILeu, Leu, Lys, Met, Phe, Thr and Val on g amino acid per 100 g total amino acid residue basis were used to determine a calculated rat PER values for both the dried hydrolysates from heads and viscera. Values were greater than 2.6 for hydrolyzed heads and 2.8 for hydrolyzed viscera. As expected, the mineral contents of raw and hydrolyzed heads reflected the removal of the bone fraction from the hydrolysate. SDS-PAGE gel electrophoreses indicated that all the major proteins present in raw heads and viscera were reduced in size to small peptides and amino acids in the corresponding hydrolysates. The percent protein solubility of hydrolyzed pink salmon head was greater than that of raw heads. Functional properties of the hydrolysate powders including: color, nitrogen solubility, fat absorption capacity, emulsifying capacity, and emulsion stability had some differences between the head and viscera hydrolysates.

In conclusion, it was possible to make dried hydrolysates without removal of fat from both pink salmon heads and viscera. Chemical, physical, nutritional, and functional properties of hydrolysates from these two salmon byproducts were determined and compared.

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L 62. MATRIX METALLOPROTEINASES IN FISH MUSCLE

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Abstract

Matrix metalloproteinases (MMPs) are calcium and zinc dependent neutral/alkaline proteinases that have specificity for extracellular matrix (ECM) components like collagens and proteoglycans. It is well known that MMPs are essential in physiological and pathological turnover of the extracellular matrix (ECM). It has also been proposed that proteolytic degradation of ECM may be involved when muscle tissue deteriorates post mortem.

In our work we have studied the gelatinolytic activity in muscle from Atlantic cod (*Gadus morhua*), Spotted wolffish (*Anarhichas minor*) and Atlantic salmon (*Salmo salar*) using gelatine SDS PAGE, gelatine affinity chromatography and enzyme inhibitors.

Gelatinolytic activity was found in the muscle of all three species, with highest activity detected in wolfish. The gelatinolytic activities in wolffish and cod muscles consisted of both serine proteinases and metalloproteinases, while only the latter was found in salmon muscle. The serine proteinases detected had molecular weight of approximately 105 kDa and 120 kDa, in wolfish and cod respectively. Activation of the metalloproteinases by *p*-aminophenylmercuric acetate (APMA) resulted in a shift in activity from high to lower molecular weight as known for mammalian matrix metalloproteinases. In all three fish species the molecular weight of the metalloproteinases were lowered from approximately 80 kDa to about 70 kDa. The metalloproteinase detected also showed high affinity for gelatine indicating the presence of a fibronectin type II domain.

Based on these experiments we conclude that there exist both matrix serine and matrix metalloproteinase-like proteinases in cod and wolfish.

Introduction

Early post mortem degradation of fish muscle may result in loss of textural properties and fillet integrity, giving a muscle that is difficult to process and hence loss of quality. It appears that this is consistent with breakdown of the extracellular matrix (ECM)(Bremner and Hallett 1985; Hallett and Bremner 1988; Ando and others 1991) and to a lesser degree by degradation of intracellular elements.(Bremner 1999; Morrison and others 2000) In addition to muscle softening during chilled storage, gaps in the fillet might also be a problem. This gaping may occur after filleting of farmed Atlantic salmon (*Salmo salar*),(Lavety and others 1988) and especially in Atlantic cod (*Gadus morhua*),(Love and others 1972) while it apparently is nonexistent in wolffish (*Anarhichas minor*).(Love and others 1969)

Matrix metalloproteinases (MMPs) have the ability to degrade several of the matrix components such as collagens and proteoglycans.(Woessner 1991) In addition to MMPs, matrix serine proteinases (MSPs) may also play an important role in degradation of ECM.(Koshikawa and others 1992) The roles of these kind of enzymes in the degradation of fish muscle during early post mortem ice storage have been paid little attention.

Materials and Methods

Crude extracts from skin free fillets from Atlantic cod, Atlantic salmon and Spotted wolffish were applied to gelatin Sepharose. Bound and unbound samples were analysed for gelatinolytic activities using gelatin SDS-polyacrylamide gels.

Results and Discussion

Gelatin SDS PAGE has been shown to be a valuable tool in investigating the presence of MMPs and other proteinases with gelatinolytic activity.(Stetler-Stevenson and others 1989; Koshikawa and others 1992; Okada and others 1992)

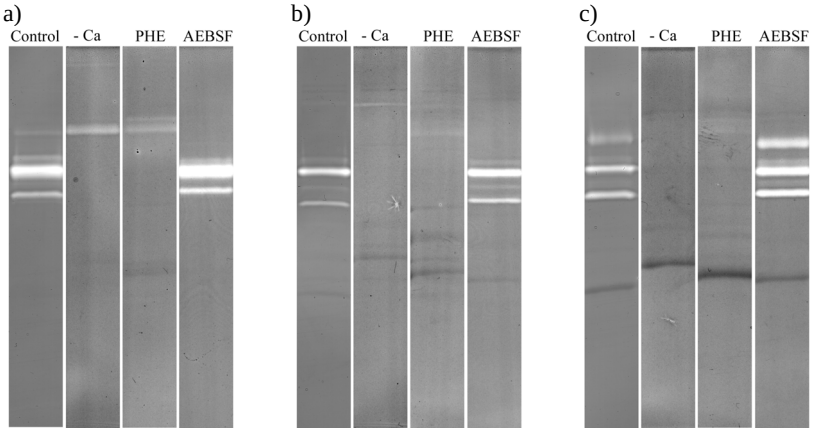

Figure 1. Zymograms of bound sample from spotted wolffish (a), Atlantic cod (b) and Atlantic salmon (c) incubated without calcium present or with calcium present in addition to proteinase inhibitor (PHE; 1,10-Phenanthroline (MMP inhibitor), AEBSF; *p*-aminoethylbenzenesulfonyl fluoride, HCl (serine proteinase inhibitor)

Matrix Metalloproteinase-2 (MMP-2, EC 3.4.24.24) and Matrix Metalloproteinase-9 (MMP-9, EC 3.4.24.35) have high efficiencies to degrade both gelatin and collagen type V. One reason for this is that they bind strongly to gelatin due to fibronectin type II domain in the catalytic region.(Nagase and others 1991; Woessner and Nagase 2000) Since the gelatinases bound to the gelatin Sepharose were completely inhibited by 1,10-phenanthroline (MMP inhibitor) and required calcium for activity we suggest that these proteinases in wolffish, cod and salmon probably are metalloproteinases with similarities to MMP-2 or MMP-9.

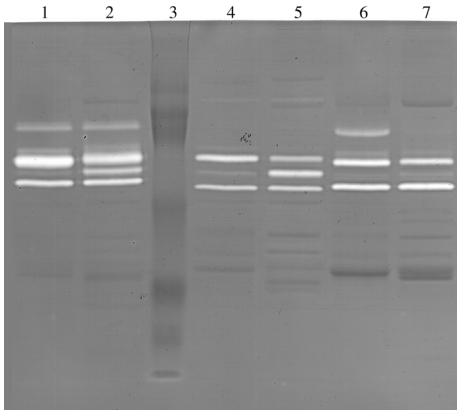

Figure 2. APMA activation of samples bound to gelatin Sepharose. Lanes 1-2; spotted wolffish, lanes 4-5; Atlantic cod, lanes 6-7; Atlantic salmon. Lanes 1, 4 and 6; controls, lanes 2, 5 and 7; APMA activated. Lane 3; prestained standards (kDa).

Additional evidence was obtained using APMA, a known activator of MMPs.(Nagase 1997) After APMA treatment all species investigated showed increased activity in some bands with subsequent decrease in other bands. The appearance of both the high and the low molecular weight gelatinases in samples not preincubated with APMA may imply that inactive and active forms of these MMP-like gelatinases coexist in the muscles of fish.

Muscle extracts from wolffish and cod contain proteinases with gelatinolytic activity which is not bound to or has low affinity to gelatin Sepharose (Fig 3; a and b, respectively). In salmon all detected gelatinolytic activities in the muscle extract were bound to the gelatin Sepharose. The non-bound activities do not require calcium for activity, and are completely inhibited by the serine proteinase inhibitor AEBSF.

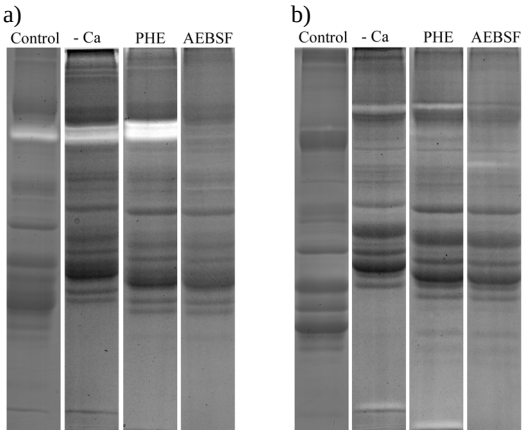

Figure 3. Gelatinolytic activities in muscle extracts from spotted wolffish (a) and Atlantic cod (b) not bound to gelatin Sepharose incubated without calcium present or with calcium present in addition to proteinase inhibitor (PHE; 1,10-Phenanthroline (MMP inhibitor), AEBSF; *p*-aminoethylbenzenesulfonyl fluoride, HCl (serine proteinase inhibitor)

Conclusions

There are both quantitatively and qualitatively differences in gelatinolytic activities in muscle of different fish species. Matrix metalloproteinase-like and matrix serine proteinases with the ability to degrade gelatin at neutral pH exists in the muscle of both Atlantic cod and spotted wolffish, while the former only can be detected in Atlantic salmon.

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L 63. SEASONAL CHANGES IN TRYPSIN AND CHYMOTRYPSIN-LIKE ACTIVITY IN VISCERA FROM COD SPECIES.

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Abstract

This work compares the trypsin and chymotrypsin-like activity in viscera from cod, saithe, haddock, tusk and ling caught in the Icelandic Sea, North Sea and South Coast of Ireland in three different seasons.

Results: Comparing data from cod caught in the Icelandic Sea reveals significant differences between seasons ($p< 0.1$) for water soluble protein, free amino acids, total protein, trypsin activity (20°C, 35°C, 50°C and 65°C) and chymotrypsin-like activity (5°C, 35°C and 65°C). Significant differences ($p<0.05$) between the five species caught in the Icelandic Sea is found for pH, water soluble protein, free amino acids, trypsin activity (50°C) and chymotrypsin-like activity (20°C, 35°C and 50°C). When comparing cod caught in the North Sea at season I and II significant differences are found only in pH. Significant differences ($p>0.1$) between cod caught at the three fishing grounds at season I is found for pH, dry matter, trypsin activity (20°C, 35°C, 50°C and 65°C). Significant differences ($p<0.05$) between cod, saithe, haddock and tusk caught in the Icelandic sea and North Sea at season II is found for dry matter, trypsin activity (5°C, 20°C, 35°C and 50°C) and chymotrypsin-like activity (65°C), and significant differences between cod caught in the Icelandic sea and South Coast of Ireland at season III is found for pH, total protein, trypsin activity (5°C, 35°C and 50°C) and chymotrypsin-like activity (5°C, 20°C, 35°C, 50°C and 65°C).

Introduction

Today's fishing industry faces increasing demands for better utilization of all the available raw material. These demands are results of both economic as well as ecological concerns, and have lead to an increasing interest in fish by-products over the past years. Annual discards from the world fisheries are estimated to be approximately 20 million tons (FAO). A rising challenge is to utilize the protein fractions from by-products for human consumption. Viscera are highly perishable fractions of the discards that traditionally have been dumped. Nevertheless it contains protein with an excellent amino acid composition and digestibility. In order to stabilize and subsequently utilize viscera natural degradation processes need to be controlled. Enzymatic activities along with microbial degradation are the most important processes deteriorating quality of viscera. The major proteolytic enzymes active at neutral pH in viscera from cod species are trypsin and chymotrypsin-like enzymes. Chymotrypsin-like enzymes include cathepsin B and calpain in addition to chymotrypsin. Knowledge of how endogenous enzymes respond to changes in temperature is of importance in order to work out optimal processes for handling. Natural variations in raw material according to species, seasons and fishing grounds also need to be characterised.

The results of this study is part of an EU-project; "Utilisation and stabilisation of by-products from cod species" (QLK1-CT2000-01017).

Materials and methods

Viscera from five species of cod were analyzed, cod (Gadus morhua) (50/60 cm, 60/70 cm and 70/80 cm), haddock (Melanogrammus aeglefinus), saithe (Pollachius virens), tusk (Brosme brosme) and ling (Molva molva). Sampling took place in February-March, April-June, and October-December 2001. Viscera were sampled from fish caught at three different fishing grounds (North Sea, Icelandic Sea and South Coast of Ireland).

Peptidase activity: Trypsin and chymotrypsin like activity was determined in crude extracts using N-t-BOC-Gln-Ala-Arg-7-amido-4-methylcoumarin and N-Succinyl-Leu-Leu-Val-Tyr-7-amido-4-methylcoumarin as substrate. The reaction mixture was incubated 10 min at respective temperatures, before reaction was stopped. Increase in emission at 460 nm (excitation 360 nm) was measured using a Perkin Elmer 3000 spectrofluorometer.

Results

Trypsin and chymotrypsin-like activity have been measured at pH 7 and five temperatures for 40 samples of viscera from cod species. All samples show highest activity at 50°C. Maximum trypsin activity expressed as increase in florescence show approximately 2000 times higher activity than maximum chymotrypsin-like activity.

Table 1: P-values for significant differences between seasons for viscera from cod caught in the Icelandic Sea.

Parameter	Season I and II	Season I and III	Season II and III	All seasons
Water soluble protein	0.028		0.000	0.005
Free amino acids	0.028	0.053	0.009	0.005
Total protein	0.089		0.018	0.031
Trypsin activity, 5°C			0.077	
Trypsin activity, 20°C	0.059	0.040		0.027
Trypsin activity, 35°C	0.095		0.016	0.098
Trypsin activity, 50°C	0.042		0.013	0.032
Trypsin activity, 65°C	0.086			0.062
Ch.trp. activity, 5°C			0.024	0.035
Ch.trp. activity, 20°C				
Ch.trp. activity, 35°C		0.076	0.017	0.052
Ch.trp. activity, 50°C	0.042		0.015	
Ch.trp. activity, 65°C	0.015	0.001	0.005	0.000

There are more significant differences between season I and II, and II and III, than between season III and I. Season III and I are in the cold part of the year, while season II is during spring/summer. Activity of trypsin and chymotrypsin-like enzymes appears to be higher during the cold seasons. Increased concentration of enzymes during cold acclimation have been proposed as the most important response to seasonal variations in temperatures (Hazel and Prosser 1974, Hochachka and Somero 1984, Ekberg 1962)

Table 2: P-values and significant differences in viscera from cod caught in the Icelandic sea, North Sea and South Coast of Ireland in season I.

Parameter	Icelandic & North Sea	Icelandic Sea & South Coast of Ireland	North Sea & South Coast of Ireland	All
pH	0.002	0.014		0.001
Dry matter	0.018	0.076		0.022
Total protein	Not an.	0.042	Not an.	Not an.
Trypsin activity, 5°C	0.098			
Trypsin activity, 20°C	0.016	0.045		0.012
Trypsin activity, 35°C	0.018			0.097
Trypsin activity, 50°C	0.031	0.073		0.032
Trypsin activity, 65°C	0.083			0.095
Ch.trp. activity, 5°C		0.095		
Ch.trp. activity, 65°C		0.003		

Viscera from cod caught in the Icelandic Sea in season I seem to differentiate from viscera from cod caught at the to other fishing grounds, especially with respect to trypsin activity.

Significant differences between the five species is found for pH, amount of water soluble protein, free amino acids, trypsin activity (50°C) and chymotrypsin-like activity (20°C, 35°C and 50°C). Khalil and others (1987) found that fish viscera differed in proteolytic activity with fish species. Hidalgo and others (1999) has also reported differences in proteolytic activity in liver plus digestive tract according to species.

Correlation's: High values of dry matter are correlated to high amounts of total protein, while high amounts of free amino acids are correlated to low values of water soluble protein. Both water-soluble protein and free amino acids are closely correlated to trypsin and chymotrypsin activity at all temperatures. Samples with low activity show high amounts of water-soluble protein, while high enzymatic activity is related to high amounts of free amino acids.

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P 49. DEVELOPMENT OF METHODS FOR EVALUATING GEL-FORMING PROPERTIES IN RESTRUCTURED FISH PRODUCTS

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Abstract

Interest in the processing of relatively low value fish cut-offs into higher value products is considerable. Current methods for utilization of fish cut-offs usually involve mincing of the fish flesh prior to reforming. During mincing structural properties are changed with subsequent loss of the textural characteristics found in intact fish flesh. We suggest that cut-offs may be reformed using dissolved fish paste as an adhesive rendering larger fish steaks with textural characteristics similar to intact fish flesh.

Our objectives were to test the adhesive properties of various pastes from different fish species and find the best composition of paste and cut-offs, for each species, to be used for reforming fish into added value products.

Traditional methods used to evaluate texture in intact fish products and surimi, are evaluation by folding, torsion tests by twisting or compression tests like TPA. None of the traditional methods were readily applicable in evaluating the adhesive properties of the reformed products. Two new methods were developed and adapted for evaluating the gel-forming properties of the final restructured fish products. The fish pastes contained different ratio of water, minced fish and salt.

The two quantitive methods developed to estimate the binding properties of the reformed fish products, were a tensile test and evaluation of stiffness.

In the tensile test, two fish slices were glued together by different types of fish paste and then fixed between two clamps and pulled apart. The max tensile strength (MTS) and the slope (Gradient1:2) were used to evaluate the gel-forming ability of the reformed fish. In the stiffness test reformed fish products of specific size were located on top of two platforms with fixed gap between them adjusted to either 5cm or 7 cm. The curvature of the sample was then evaluated by recording the distance from the sample to base flooring the two platforms.

The results showed that the tensile test and the stiffness test could be used to evaluate gel-forming ability of different reformed fish products.

Introduction

The objects of this work were to develop methods to evaluate fish pastes to be used as adhesives when restructuring fish cut-offs into fish steaks.

Different levels of salt, minced fish and water were used to define the best composition of a fish paste. Four different species of white fish (cod, blue whiting, haddock and pollack) were tested to improve the knowledge of the gelling or binding properties of pastes from different fish species. Two new methods were developed and adapted for evaluating the gel-forming properties of the final restructured fish products.

Materials and methods.

Raw material.

Fish for raw material was caught by net at the east coast of Iceland and transferd to the Icelandic Fisheries Laboratories (IFL) on ice. The fish was wrapped in PVC film and kept in ice.

Tensile test to analyse the gel-forming properties of the fishglue and the reformed fish products.

1. Preparation of fish slices.

The fillets without skin and bones were cut into slabs of 3cm x 3 cm in size and then quickly frozen at –24°C for 24 hours, the frozen slabs were carefully tempered to -3°C before slicing. A panasonic electric meat slicer was used to slice the fish slabs into approximately 2 mm thick slices and approximately 30 mm x 30 mm wide. The slices were placeed loosely on a plate and kept in ice at 2°C before testing. The plate was tightly wrapped with layers of PVC plastic film to prevent dehydration of the slices.

2. Preparation of fishpaste.

The weighted fish minces were deposited into a mixing bowl, 20% added water and 2% added salt of the initial minces weight were accurately weighed and mixed throughly in a big beaker. The well mixed brine was deposited into the mixing bowl in a Stephan UMC 5 electronic mixer (Germany) set at 24 (1/min*100) speed with the fish minces for 3 min at room temperature.

3. Preparation of samples for tensile test.

Two fish slices were glued to two separate wood T-holders with Turbo super gel, and allowed to set. After preparation, the pre-mixed fishglue was spread carefully between the two fish slice surfaces, and the assembly was carefully adjusted to be parallel.

The glued samples were then placed into a plastic plate, wrapped with PVC film and frozen at 0°C and stored for 1 week before the tensile testing.

4. Tensile test.

The frozen samples were thawed at 2°C over night, and analysed both before and after cooking. The tensile sample was held by a special set of grips, holding the sample by the two T-holders, then the sample was pulled apart at a speed of 0,8mm/s in a TA.XT2® Textural Analyser. The maximum tensile strength (g) (MTS) was recorded as the force required to separate the glued slices.

Stiffness test to analyse the adhesiveness and binding properties of the reformed fish products.

1. Preparation of fish trimmings.

Fish fillets were manually cut into 1cm x 1cm x 1cm cubic trimmings, and packaged into PVC bags, and kept in ice in a cool room at 2°C until analysis.

2. Tumbling and reforming.

Fish trimmings and mixed fishpaste were tumbled together at room temperature under vacuum for 30 min, the ratio of fish-paste to fish trimmings was 1:4. Tumbling was conducted under vacuum (–0,6 bar) at a tumbling speed of 20 rpm in a Dorit Vacuum-Marinator Tumblers MGH-20 (Food processing Equipment Ltd, Switzerland) After tumbling, the trimming-paste mixture was immediately deposited into rectangle carton boxes (size is 22cm × 7,5cm × 3,7cm). All reformed fish samples were frozen and stored for further analysis.

3. Stiffness test.

Stiftness test was a new method to analyse the gel-forming properties of fish glue. In the stiffness test reformed fish products of specific size were placed on top of two platforms with a fixed gap between them, adjusted to either 5cm or 7cm. The curvature of the sample was then evaluated by recording the distance from the sample to the base flooring of the platform.

The falling percent (%) was used to express the different gel-forming properties of the various fishglues in the reformed samples.

Falling percent (%) was calculated from the distance (cm) measured from the platform to the sample and expressed as a percentage of the heigth.

The lower the falling percent (%), the stronger the binding properties of the reformed fish sample.

Results and discussions

These white fish species cod, haddock and pollaok and 8 types of reformed fish samples (CCI, CCII, CHI, CHII, CPI, CPII, HH and PP) were restructured into fish slabs or steaks of a set size.

The max tensile strength (g) [MTS] varied from 450g to 1380g for different samples. The haddock-haddock and pollack-pollack reformed fish steaks had better binding properties than the cod-cod reformed fish steaks, which indicated that the pollack and haddock species had better gel-forming properties than the cod species. The binging increased in the following sequence: CC<CH<CP<HH<PP.

The [FP] for cooked samples with a 5cm gap varied from 1,6% to16.13%. All the reformed samples had lower FP(%) value (1,6%-2,69%) than the intact fillet group (3,23%-16.13%) and control trimming group (3,23%-7,53%) after cooking, indicating that reformed fish samples had better binding properties than the control.

Conclusion

The results demonstrate that both the tensile and the stiffness test may be used to evaluate the adhesiveness and gel-forming properties in reformed fish products. The stiffness test was both inexpensive, very rapid, easy to perform and highly illustrative. The test should be easily adapted to quality control in a food processing environment. Analysis of the adhesive properties of the fish pastes was better controled in the tensile test, giving results with higher accuracy but the test was more complex and is likely better suited for research.

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P 50. THE USE OF RESPONSE SURFACE METHODOLOGY TO EVALUATE SURIMI, FAT AND WATER CONTENT IN A LOW/NO ADDED PORK SAUSAGE FORMULATION

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Abstract

Surimi, a concentrate of myofibrillar proteins obtained after mincing and washing fish flesh, is valued primarily for its gelling properties. The objective of this study was to employ response surface methodology (RSM) for simultaneous analysis of the effects of added levels of surimi, fat and water on the physical, textural and sensory characteristics of fresh pork sausages. Three optimum recipes, R1 (25.3% surimi, 22.2% fat, 12.7% water, 25.3% pork), R2 (12.2% surimi, 5.5% fat, 38.7% water, 33.2% pork) and R3 (25.3% surimi, 6.3% fat, 28.5% water, 25.3% pork) identified from RSM plots were evaluated against a full-fat commercial control (R4). Sausages were evaluated for composition, texture, colour and sensory attributes. Optimised recipes had lower ($p<0.001$) fat and protein contents compared to R4. Hardness values of R1 were not significantly different to R4, however hardness of R2 and R3 were lower ($p<0.001$) than R4. Sensory analysis (12 untrained panellists) R1, R3 and R4 scored >65% and R2 scored >55% for colour, chewiness, texture, flavour and acceptability. Surimi (25%) may be used to reduce fat content in pork sausages without adversely affecting texture, visual appearance or organoleptic properties.

Introduction

Consumer preference for alternative healthier meat type products is stimulating the research and development of meat systems with altered compositional profiles (Cofrades *et al*., 2000). Moreover, the consumer interest in the development of meat analogs using alternative protein sources is gaining in popularity. Surimi is a Japanese term, which defines a concentrate of myofibrillar proteins obtained after mincing and water washing of fish flesh (Park & Morrissey, 2000). Surimi is light in colour, has a bland odour, low in fat and high in myofibrillar protein and extremely functional due to the unique gelling properties of these myofibrillar proteins and these qualities make surimi an ideal functional ingredient for fabricating new food products (Lanier, 2000). The objectives of this study were to assess the texture and organoleptic properties of breakfast pork sausage using response surface methodology with varying levels of whiting surimi, fat and water, and to compare the optimum formulations identified to that of a standard full-fat commercial sausage.

Materials ad Methods

Response surface methodology (RSM) was used to study the simultaneous effects of three compositional variables, namely; water (10-35%), fat (5-30%) and surimi (0-40%). The experiments were based on a central composite rotatable design (Cochran & Cox, 1957). Five levels of each factor or variable (surimi, fat and water content) were provided in accordance with the principles of the central composite design (Lyons *et al*., 1999). Whiting surimi was produced using traditional methods. The pork-surimi sausage mixtures were prepared according to the formulations obtained from the composite design. The batter was filled into cans (100±0.5g) which were sealed and pasteurised (80°C for 2 h) or sterilised (121°C for 15 min), and were evaluated for texture, colour and water holding capacity. Optimised recipes, which were determined from three-dimensional plots and contour plots, were evaluated against a commercial full-fat sausage (control). Textural properties were evaluated using the SMS Texture Profile Analyser (TPA). Total protein content was determined using the Kjeldahl method. Moisture and fat content was evaluated by CEM analysis. The ash content of samples was determined by mineralisation at 550°C. CIE L, a and b values were measured using a Minolta CR300 colorimeter. An in house panel (untrained but were experienced in sensory evaluation of various other food products) consisting of 12 people performed sensory analysis. **Results and Discussion**

RSM three-dimensional plots and contour plots were used to extrapolate the optimum level of the three variables (surimi, fat and water). Three optimum recipes R1 (25.32% pork, 25.32% surimi, 22.15% fat, 12.66% water, 12.66% rusk, 1.9% seasoning), R2 (33.21% pork, 12.17% surimi, 5.53% fat, 38.71% water, 9.04% rusk, 1.9% seasoning), and R3 (25.32% pork, 25.32% surimi, 6.33% fat, 28.48% water, 12.66% rusk, 1.9% seasoning) were determined from three-dimensional and contour plots and these were evaluated against a full-fat commercial control sausage product (R4). The composition of optimised recipes are presented in Table 1. Optimised recipes had lower ($p<0.001$) protein and fat content compared to commercial full-fat control sausages. Ash content of the optimised recipes were lower ($p<0.001$) than controls. Moisture content for R2 and R3 were higher ($p<0.001$) than

R4 with R2 having the highest ($p<0.001$) moisture content of samples assessed. R2 (15.92N) and R3 (17.22N) had lower ($p<0.001$) hardness when compared to R1 (24.70) and R4 (23.16N) (the commercial full-fat control). Cohesiveness of R1 (0.52) and R3 (0.47) was not significantly different to R4 (0.53), however cohesiveness of R2 (0.32) was lower ($p<0.05$) than R4. Thus, 25% surimi in combination with 22% fat and 12% water can be used in sausage formulations without significantly affecting textural properties of the products. The CIE 'L' values (ranged from 69 to 73) of R1, R2 and R3 were not significantly different to those of the control (R4). There was also no significant difference in 'a' (ranged from 14 to 17) or 'b'values (ranged from 12 to 17) of raw optimised sausage formulations (R1, R2 and R3) and the control (R4). Sensory analysis (12 untrained panelists) results showed that R1, R3 and R4 scored >65% and R2 scored >55% for colour, chewiness, texture, flavour and acceptability. R2 scored lowest for all sensory attributes tested. These results indicate that potentially surimi could be used as a meat extender or fat replacer in sausage formulations.

Table 1. Composition of optimised formulations for pork-surimi sausage

%	R1	R2	R3	R4
Protein	10.60±0.32 [a]	9.96±0.18 [b]	9.77±0.27 [b]	12.97±0.14 [c]
Fat	16.81±0.56 [a]	5.43±0.47 [b]	5.13±0.36 [b]	18.7±0.98 [c]
Moisture	54.03±0.11 [a]	71.74±0.29 [b]	68.29±0.52 [c]	55.54±0.83 [d]
Ash	2.17±0.05 [a]	1.71±0.06 [b]	2.04±0.06 [a]	2.85±0.06 [c]

[a, b, c, d] Values with different letters within a row indicate statistically significant differences ($p<0.05$).

Conclusions

Three optimum recipe formulations were identified from initial RSM analysis of pasteurised, sterilised and fried comminuted pork-surimi systems. Levels of 25% surimi in combination with 6.3% fat and 28.5% water or 22% fat and 12.6% water may be used in the manufacture of pork sausage product without adversely affecting its flavour, acceptability and consumer preference or its visual colour and visual acceptability. In summary his study identifies the potential benefits of surimi (25%) as a meat extender or a fat replacer in pork sausages.

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P 51. ALKALINE PROCESSING AND USE OF VANE TEST FOR QUALITY CONTROL OF SURIMI AND SURIMI SEAFOODS

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Abstract

A novel process methodology, utilizing isoelectric precipitation following high pH (alkaline) solubilization, was explored for isolating functional protein from fish and other meat species (Hultin and Kelleher, 2000, 2001). Optimum pH ranges for both solubilization and reprecipitation were identified. Fresh, unfrozen meat was found to yield more functional protein than previously frozen meat, but still produced acceptable gels by the alkaline process. Gel properties of protein recovered by this method were superior to those recovered by a traditional surimi process, and yielded considerably higher protein recovery. A novel method of assessing gel-forming ability of gelled proteins based on vane application to stacked sheets was developed and shown to correspond to results obtained conventionally by torsion on cylindrical-shaped samples. The latter has special applicability to online testing of surimi seafoods in mid-production.

Materials & Methods

Raw Materials and Processing

Atlantic croaker (*Micropogon undulatus*) mince was made using a Yanagiya mini belt deboner. The mince was stabilized with a cryoprotectant mixture (10% sucrose and 0.3% sodium tripolyphosphate), vacuum-packaged in oxygen impermeable bags and held frozen at –50C. Conventional surimi was made by washing frozen, stabilized mince with cold water (<5°C) at a ratio of 1:3(w/v), then centrifuged to dewater, blended with the same cryoprotectant mixture as before and frozen at –50C. Protein isolate was made from frozen, stabilized mince by homogenizing with water at a 1:9 (w/v) ratio, with pH subsequently adjusted to pH 9, 10, 11, or 12, respectively, using 1N NaOH. The solubilized protein suspensions were centrifuged to yield three main fractions: a sediment containing insoluble membrane, skin, bones, etc.; a soluble protein layer, and a fat layer. Soluble proteins decanted and separated from fat by filtering through cheesecloth, then precipitated by adjustment of the solution to near the isoelectric point (about pH 5.5), or in one case to the pH range 5.5-7.0, using 1N HCl. Precipitated proteins were collected by a second centrifugation, excess water water was decanted, then the proteins blended with the previous cryoprotectant mixture and frozen at –50C.

Composition and Functionality

Moisture contents were measured by oven drying (AOAC, 1990). Protein contents were measured on soluble samples by the Bio-Rad method (Bradford, 1976) and on minces using a Perkin-Elmer PE 2400 CHN Elemental Analyzer. Viscosity of protein sols was measured on a Brookfield viscometer. Gels were prepared with 2% NaCl, 78% moisture content at pH 7.1 (upon addition of 1 N NaOH). Prepared pastes were cooked either in stainless steel tubes (19 mm i.d.) or as a thin (4.2 mm) sheet by heating in a water bath at 90^{o}C for 20 min. Cylindrical gels were tested by torsion (Hamann, 1983, 1991) and sheet gels by a novel vane test which utilized a four-bladed (5.3mm dia., 31mm high) vane rotor attached to a Brookfield viscometer. Three sheets of thin surimi gel were stacked and clamped, then the vane was inserted, extending out of the stacked gels at each end, and rotated at 2.5 rpm. Data for yield stress and corresponding angular deformation (rad) were collected (Dzuy and Boger 1983, 1985; Steffe 1996). Color of cooked gels was measured on a Minolta Chroma Meter CR 300 and whiteness index defined as L*-3b* (Park 1994).

Results & discussion

Protein solubilization effects: Viscosity of homogenates decreased with increasing solubilization pH. Holding at any pH above 9 for up to 24 h additionally reduced viscosity, but not substantially. Low viscosity assists in removing labile phospholipid-rich membranes during centrifugation. Adjusting to pH 10 or above and holding 30 min resulted in more complete solubilization of muscle proteins; solubilization of proteins ranged 57, 84, 89, and 91% for pH 9, 10, 11 and 12, respectively. When these were precipitated at pH 5.5 the yields of protein ranged 39, 72, 82, and 87% for the respective solubilization pH values (9-12), based on initial protein content of meat; this compares to only 60% protein recovery by conventional water washing. Gels prepared from these isolates were stronger and more deformable from isolates prepared by solubilizing meat at pH 10.5 or above, with values almost double those obtained by solubilization at lower pH, or by conventional water washing.

Precipitation pH effects: Precipitation and centrifugation above pH 5.7 reduced protein recovery; removal of water was difficult and solution viscosity was higher above this pH. Strongest gelation was noted in protein isolates

precipitated at pH 5.5. Gels from isolates precipitated at pH 5.9 showed a 50% reduction in strength, while those from isolates precipitated at pH >6.0 were very weak and sponge-like in appearance. Strain at failure was not affected by pH of precipitation up to pH 5.9.

Fresh vs. Frozen Fish: The gelling properties of isolate prepared by the solubilization/precipitation process at pH 10.5/5.5, respectively, were compared using fresh vs. frozen, stabilized mince as raw material. A similar comparison was made for gelling properties of surimi made by the conventional washing method. Large differences were seen between treatments with the latter method of surimi manufacture (stress/strain of 12.7/2.6 for fresh vs 5.6/1.6 for frozen). Differences between treatments for the alkaline process isolate were less but still evident (stress/strain of 18.8/2.1 for fresh vs 16.2/2.0 for frozen), indicating the alkaline method can produce much stronger gels, even when frozen mince is the starting raw material.

Torsion vs. Vane: Generally vane measurements of stress and strain paralleled one another, indicating that measurements made on sheet-produced surimi generally predict those of torsion made on conventional cylindrical samples. Sheet samples can be prepared by faster heating rates, eliminating protease effects in gel softening; additionally product produced during manufacture of sheet-type surimi seafoods can be analyzed directly for textural properties by this approach.

Color of Gels: Isolate gels prepared by solubilizing meat at pH 10 or 11, and precipitated at pH 5.1-5.9, all had similar whiteness (about 37-39). These were whiter than when solubilization occurred at pH 9 or 12, but not as white as the precipitates at pH 6 and above. Whiteness of surimi prepared by the conventional washing method was greater still (about 57), indicating that the isolate still contains heme proteins that are removed by conventional washing.

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P 52. STABILIZATION OF FUNCTIONAL PROTEINS RECOVERED FROM HERRING (*CLUPEA HARENGUS*) FILLETS BY AN ACID SOLUBILIZATION PROCESS

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Abstract

Recently, procedures were developed to recover functional proteins from low value muscle sources such as herring. Minced muscle is homogenized with water (1:9) after which the muscle proteins are solubilized at low pH (2.7) or high pH (10.8). Oil and insoluble materials like skin, bones, and, under favorable circumstances, even cellular membranes can be separated from the solubilized proteins by centrifugation. The soluble proteins are precipitated at pH 5.5 and recovered by a second centrifugation. Both the acid and alkaline processes give high yields of herring proteins with good functionality. However, unless there is a 100% removal of lipids and hemoproteins, the acid process can induce some lipid oxidation problems. This is since acid increases the pro-oxidative properties of haemoproteins.

This work was conducted to evaluate how modifications of the process settings and additions of antioxidants could prevent lipid oxidation during acid processing of skinned herring fillets and during ice storage of the final herring protein isolates.

Processing parameters evaluated were: pre-wash of the mince, exposure time to pH 2.7, and the inclusion or exclusion of the first centrifugation. Antioxidants tested were: eythorbate, STPP, EDTA (0.2%) and milk proteins (4%). The three former were added in the pre-wash or during the homogenization step while milk proteins were added to the final precipitate. All isolates were stored in Erlenmayer-flasks on ice while following odor, TBARS and color changes.

A pre-wash or varied exposure time to pH 2.7 did not affect lipid oxidation. Excluding the first centrifugation step gave ~40% higher levels of oxidation products in the isolates. Erythorbate alone or in combination with STPP/EDTA efficiently prevented lipid oxidation if added in the pre wash or homogenization step. No further stability was gained from adding antioxidants in both these steps.

Introduction

Approximately 90% of the estimated 2.5 million metric tons of herring that are captured are directed towards non-human consumption as fish-meal and fertilizers. Isolation of pelagic fish proteins for food production would be a more responsible way of using a nutritious and abundant raw material. However, the seasonality, small size and unfavorable composition of herring have made it difficult to extract stable and functional proteins for food applications (Okada, 1980; Opsvedt, 1985).

Recently, new procedures were developed to recover functional proteins from low value muscle sources such as herring (Hultin & Kelleher, 1999; 2000ab; 2001). The principle is that minced muscle is homogenized with water (1:9) after which the muscle proteins are solubilized at low pH (≤3) or high pH (≥10.5). Oil and insoluble materials like skin, bones, and, under favorable circumstances, even cellular membranes can be separated from the solubilized proteins by centrifugation. The soluble proteins, forming a supernatant between the floating oil layer and the sediment, are precipitated at pH 5.5 and then recovered by a second centrifugation. Using light muscle from herring caught along the coast of New England (Undeland *et al*., 2002), 74% and 68% of the total proteins were recovered with the acid and alkaline process, respectively. The acid- and alkali-produced proteins had good gelling capacity and color. Seventy % of the muscle lipids (2.4%, ww), and 50% of the phospholipids were removed.

It has been found (Kristinsson, 2003) that unless there is a 100% removal of lipids, the acid process can induce some lipid oxidation problems. This is thought to be since the acid increases the pro-oxidative properties of haemoproteins in the muscle. Possible pathways for acid haemoprotein activation are deooxygenation, autoxidation, subunit dissociation and heme-group/Fe-liberation. To overcome these problems, one way would be to reduce the level of haemoproteins in the mince e.g. via the introduction of a pre-wash. Other ways are to reduce the exposure time to low pH, and to add antioxidants. Based on previous findings (Kelleher *et al*., 1992; Richards *et al*., 1998), antioxidants should be added *early* in the process, and should inhibit the pro-oxidative properties of both heme-bound Fe^{3+} and low molecular weight (LMW) Fe^{2+}. Good results have also been obtained with compounds that can scavenge radicals and/or volatile oxidation products (Lee and Liam, 2001).To what extent the high-speed centrifugation step affects the oxidative stability of the protein isolate is another point of high interest; particularly from an economical aspect.

The aim of this work was to evaluate how the oxidative status (TBARS) of herring protein precipitates were affected by: (i) pre-washing herring mince, (ii) varying the exposure time to low pH, (iii) including or excluding a high speed centrifugation step, and (iv) adding antioxidants (erythorbate, STPP, EDTA and milk proteins) at various stages of the acid process (pre-wash, homogenization or final precipitate). The protein isolation process was done in lab scale. Changes in TBARS and odor were also followed during ice storage of the protein isolates.

Materials and Methods

Skinned fillets from herring (*Clupea harengus*) caught in Kattegat, November 2002 were ground and frozen in 120 g portions at -80°C. The fresh mince had a pH of 6.6 and a moisture content of 72%. The mince was thawed for 30 min under cold running water and then homogenized for 1 min with 9 volumes of ice-cold distilled water (DW) using an Ultra Turrax homogenizer. Two of the 120 g-portions (# 3 & 4 in **Table 1**) were first pre-washed in 3 vol of ice-cold DW (1 min stirring, 15 min settling, and dewatering in a sieve). Proteins in the homogenate were solubilized by drop-wise addition of 2N HCL until reaching pH 2.7.The protein suspension was then centrifuged for 20 min at 10,000×g, and the supernatant was separated from the floating oil layer by filtering these two phases through double cheesecloth. In samples were the high-speed centrifugation was excluded, the homogenate was instead held for 75 min at the low/high pH (i.e., time required for the centrifugation and filtering steps to be completed). The soluble proteins of the non-centrifuged and centrifuged homogenates were then precipitated at pH 5.5 and collected via centrifugation at 6,000×g (20 min). pH, moisture and TBARS-values were recorded for the precipitates prior to freezing them at -80°C (**Table 1**). The TBARS method was that of Lemon (1975), and data were expressed as µmol MDA/kg tissue.

To study the effect of ice storage on lipid oxidation, thawed precipitates were adjusted to 91% moisture with ice cold DW after which the pH was raised with 1 and 0.1 N NaOH to 6.55. Streptomycin (200 ppm on a moisture basis) was added manually to prevent bacterial growth. Two samples (#16 & 17, **Table 1**) were fortified with 4% (w/w) Alapro milk proteins. 25 g of each protein sample was then flattened out in the bottom of screw-capped glass Erlenmeyer flasks and were stored on ice for up to 17 days. The sample thickness was about 6 mm. At regular intervals, 7 trained panelists sniffed the headspace above the samples by uncapping the flasks. Panelists concentrated on detecting odors described as “painty”, “ocean”, “fishy” and “mineral” using a scale of 0 to 10, with 10 being the strongest. In this paper, we only report data on “painty odor”. At regular intervals, 1g-sample "plugs" were also taken out from the precipitate and were frozen in aluminum foil at –80°C. To confirm the sensory data, selected “plugs” were taken for TBARS-analysis.

Results and Discussion

Table 1 summarizes the preparation characteristics of the 17 isolates made, their initial pH-values, moisture contents and TBARS-values. The initial TBARS of the muscle was 8 µmol MDA/kg tissue, which was not affected by pre-washing in either water or an antioxidant solution (0.2% erythorbate and 0.2% STPP in water). After acid processing of the unwashed or water pre-washed minces, the precipitates contained 25 and 40 µmol MDA/kg when the high-speed centrifugation at 10, 000 × g was included and excluded, respectively. These findings indicate that extensive lipid oxidation took place during the acid process, most likely due to the pro-oxidative action of acid-activated haemopoteins (Richards & Hultin, 2000; 2002; Undeland *et al*., 2001; 2003). The data also show that a 1:3 water-pre wash did not reduce oxidation, but that 38% of the TBA-reacting oxidation products (and/or compounds contributing to their formation) were removed into the floating oil layer and membrane containing sediment using a high-speed centrifugation.

When including high-speed centrifugation in our lab-scale process, the herring muscle homogenate was exposed to pH 2.7 for 75 min. To evaluate if shortening of the 75 min eposure at pH 2.7 that was needed to complete high-speed centrifugation and filtration, experiments were carried out were the exposure time was shortened to 30 min and 4 min. In these experiments (#13-15, **Table 1**), the high-speed centrifugation step was excluded. TBARS of the precipitates were 50-62 µmol MDA/kg using 4, 30 and 75 min exposure time at pH 2.7, showing that the damage caused by the acid, e.g. on hemoglobin was very fast.

When adding 0.2% erythorbate and 0.2% STPP into the pre-washing water, TBARS of the final protein precipitate was reduced from 40 down to 3 µmol MDA/kg. Adding the same antioxidant mix also into the homogenization water**,** did not improve the stability further. Low TBARS (5 µmol MDA/kg) were also found in precipitates from unwashed mince when adding 0.2% erythorbate and 0.2% STPP in the homogenization water. These data clearly show that the combination of a LMW- Fe^{2+} chelating agent, STPP, and a heme- Fe^{3+} reducing agent, erythorbate, efficiently prevented oxidation from taking place during acid processing of herring proteins. Ascorbic acid at the levels currently studied (Kelleher *et al*., 1992) is also known to be a radical scavenger. Thus, the added erythorbate, i.e. iso-ascorbic acid, might have possessed such effects as well in our process.

It was found that the 0.2% STPP drastically reduced the protein solubility at pH 2.7, which reduced the protein yield in the high-speed centrifugation step. It was therefore tested to use 0.2% erythorbate alone, which gave a precipitates with 10 µmol MDA/kg. Another strategy was to add the 0.2% erythorbate in the homogenization, and the 0.2% STPP *after* the high-speed centrifugation, i.e., directly into the collected supernatant. This gave precipitates with 9 µmol MDA/kg. Thus, to not have the STPP present during acidification and centrifugation reduced the protection somewhat. A third way tested to not reduce yields was to replace STPP by EDTA, another well-known metal-chelator. When added in the homogenization step at a 0.2% level along with 0.2% erythorbate in the homogenization, TBARS of the precipitate were 5 and 7 µmol MDA/kg with and without the high-speed centrifugation, respectively.

Table 1: The protein precipitates evaluated, including their pH, moisture content (Mc), weight and TBARS-values. The minced herring used had pH 6.6, 72% moisture and 8 μmol MDA/kg. (Ao=antioxidants, HS=high speed centrifugation, Min.=minutes, +ao= 0.2% erythorbate and STPP).

#	Pre-wash	Ao in Homogen-ization	HS Centr	Min.at pH 2.7	Milk prot.	pH	Mc (%)	TBARS (μmol MDA/kg)
1	No	No	No	75		4.6	91	40
2	No	No	Yes	75		4.7	87	25
3	Yes	No	No	75		4.8	88	42
4	Yes	No	Yes	75		4.7	91	24
5	Yes (+ao)	Ery+STPP	No	75		4.9	76.5	5
6	Yes (+ao)	No	No	75		4.2	87	3
7	No	Ery+STPP	No	75		4.8	80	5
8	No	Ery+STPP	Yes	75		4.6	90	6
9	No	Ery	No	75		4.3	87	10
10	No	Ery+EDTA	Yes	75		4.7	90	5
11	No	Ery+EDTA	No	75		4.4	86	7
12	No	Ery+STPP*	Yes	75		5	87	9
13	No	No	No	4		4.5	84	56
14	No	No	No	30		4.7	85	62
15	No	No	No	75		4.6	86	50
16	No	No	No	75	Yes	4.6	83	56
17	No	Ery+EDTA	No	75	Yes	4.4	83	7

*STPP was added after the high-speed centrifugation.

It was evaluated how well the process-modifications and the addition of different antioxidants at various stages of the process affected TBARS and "painty" odor development during ice storage of the isolates. Painty odor has previously been found to correlate strongly to TBARS development in washed cod mince fortified with trout hemoglobin (Richards & Hultin, 2000; 2002; Undeland *et al*., 2001; 2003). To be able to compare all precipitates under equal conditions, initial variations in pH and moisture were evened out.The precipitate with the highest initial moisture content, 91% (#1, **Table 1**), became the standard for the other samples. The pH was adjusted to 6.55 ±0.05, the value commonly found in post mortem herring tissue.

At the beginning of the storage, four of the antioxidant-fortified isolates had low painty odor values (<2), and stayed below 2 throughout 17 days on ice. Three of these were treated with erythorbate and EDTA in the homogenization step, with and without the inclusion of a high-speed centrifugation. One sample had STPP instead of EDTA. Samples with erythorbate and STPP added both in a pre-wash and homogenization, as well as the sample were STPP was added *after* the high-speed centrifugation, stayed below an intensity of 2 for 9 days on ice, but then rapidly increased. Samples to which erythorbate alone had been added in the homogenization step, and were erythorbate plus STPP had been added in the pre-wash but not in the homogenization, started at paintiness values around 3, and then immediately started to increase. Thus, EDTA seemed to be a more antioxidative chelator than STPP, and a erythorbate alone was not as efficient as when combined with a chelator. Also, although antioxidant additions in the pre-wash alone was enough to obtain good stabilization during the process, it did not yileld an isolate with good storage stability.

Precipitates with no antioxidants added had initial paintiness values between 2 and 5. The higher values were for "regular" precipitates that were not subjected to a high speed centrifugation. In these samples, no further changes were seen during the storage, while the painty odor intensity increased in precipitates with lower initial values. This indicates that the non-centrifuged samples had most likely reached their "peak" in painty odor (Undeland *et al*; 2001; 2003) already before the storage trial started. The data also show that the high speed centrifugation reduce, but not eliminate lipid oxidation during short term storage. The highest painty odor intensity detected, 7, was found in the precipitate made from pre-washed mince without the use of a high-speed centrifugation. This could indicate that the net-effect of a water-wash was dominated by removal of natural antioxidants rather than removal of pro-oxidative heme-proteins (Undeland *et al.*, 2003). Four % milk proteins were added to one antioxidant-stabilized protein precipitate (0.2% erythorbate plus EDTA, no high-speed centrifugation), and one unstabilized precipitate (no pre wash, no high-speed centrifugation). There was no detectable effect from the milk proteins on painty odor in these samples. Lee and Lian (2001) reported that milk proteins can act both as radical scavengers and as sinks for volatile oxidation products. We will evaluate this capacity further using samples that initially are low in oxidation products, but that tend to oxidize during the storage. The samples selected here were either "already oxidized" or did not oxidize at all. In agreement with the TBARS-formation during the process (**Table 1**), there were no difference in painty odor development during storage whether the exposure time to pH 2.7 was 4, 30 or 75 min.

Conclusion

This study shows that TBARS develop during isolation of proteins from herring mince with an acid solubilization process. Reduced exposure time to pH 2.7, or inclusion of a 1.3 pre-wash with water did not

minimize oxidation. Including high-speed centrifugation reduced TBARS-levels in the precipitates by 37%. Process-induced oxidation was efficiently prevented by early addition of a metal reducing agent (erythorbate) and a metal chelator (STPP or EDTA). To gain good storage stability, antioxidant had to be added in the homogenization step, EDTA here seemed more efficient than STPP.No effect was detected from adding milk proteins (4%) to the precipitate, but this will be further evaluated.

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P 53. THE FUNCTIONAL AND PHYSICAL PROPERTIES OF PROTEIN INGREDIENTS MADE FROM MUSCLES OF WARM WATER FISH SPECIES

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Abstract

Acid and alkali-aided processing of fish muscle has been found to successfully compete with surimi processing to produce protein isolates (PI) from fish muscle of good functional properties. Many warm water species have not yet been evaluated in terms of these processes.

The objective was to compare PI's made with acid and alkaline-aided processes with conventional surimi produced from five fish species of warm and temperate waters.

Catfish, tilapia, croaker, mullet and Spanish mackerel were selected for this study. Solubility and viscosity of the fish muscle proteins as a function of pH was studied to identify pH values for acid and alkali-aided processing. A conventional process was used to produce surimi. Protein isolates were prepared by solubilizing the muscle proteins at low (2.5) and high (11) pH, collecting them via centrifugation and precipitating them at pH 5.5. Protein recovery and lipid reduction of surimi and PI was calculated and protein composition assessed by SDS-PAGE. Gelation properties of surimi and PI (pH 7.0 and 500 mM NaCl) were tested in a rheometer. Color stability and lipid oxidation of PI's and surimi was tested during and after the processing using a digital machine vision system and TBARS analysis, respectively.

Greatest protein solubility and lowest viscosity was obtained below pH 3 and above pH 10.5. The acid and alkali-aided processes gave greater protein recoveries than the surimi process and led to significantly more reduction in lipids than surimi processing. The PI from the alkali process gave the strongest gels while acid PI's gave very poor gels, except for croaker. Protein electrophoresis did indicate hydrolysis at acid pH may play a role in the poor gelation for the acid PI's. Results showed that samples differed in their color properties. Alkali processing in general gave a product of better color compared to acid process due to removal of heme proteins (e.g. higher whiteness (L*)). The alkali process also led to significantly less lipid oxidation compared to the acid process, and was comparable in stability to surimi.

These results show that the PI from the alkali-aided process could successfully compete with surimi processing of fish from warm and temperate waters. More work on the molecular level is needed to understand the differences seen with the acid and alkali-process.

Introduction

The demand for quality fish protein in the world is growing at a faster pace than the supply of traditional fish species which is leading to serious depletion of many important commercial species (Hultin and Kelleher, 2000; Kristinsson and Rasco, 2000). This situation has created a great need to more responsively utilize our limited aquatic resources. Recent efforts have focused on utilizing fish species and fish processing byproducts not previously utilized as primary source of human food. Using conventional processes to produce functional products from these raw materials has been met with many problems due in part to seasonality and small size of some species, abundance of oxidatively unstable lipids and pro-oxidants (especially heme proteins), unstable muscle proteins of low functionality and poor extractability and relatively high proteolytic activities (Okada, 1980; Hultin, 1994; Hultin, 2002), to name a few relevant examples. To address these problems two processes have been developed to economically produce functional protein isolates from fish sources of low value (Hultin and Kelleher, 1999; Hultin et al., 2000). These processes (acid-aided and alkali-aided solubilization/precipitation processes) utilize the pH dependent solubility properties of fish muscle proteins for their separation and recovery from other components of muscle not desirable in a final product.

Proteins isolated from several cold water species using these processes have been found to have good functional properties compared to proteins isolated from conventional surimi processes (Kelleher et al., 2001; Choi and Park, 2002; Undeland et al, 2002; Kristinsson and Hultin 2003). A number of fish species harvested in warm regions of the world have a great potential to be increasingly or more properly utilized for their proteins. For this reason it was of interest to investigate how the two processes would compare to conventional processes in protein recovery and protein function.

Materials and Methods

Fresh (never frozen) channel catfish, tilapia, Atlantic croaker, mullet and Spanish mackerel were selected for the study, all species of commercial importance to the southeast USA. Upon arrival to the laboratory the fish were filleted, skinned and processed within 2 hours. The solubility and rheological properties of the fish proteins were

studied at pH 1.5-12. Protein isolates were produced by homogenizing ground fish muscle and cold (4°C) water (1:9 ratio) followed by either lowering the pH to 2.5 (acid-aided process) or increasing the pH to 11 (alkali aided process). The material was then centrifuged at 10,000 x g for 20 min at 4°C and the middle phase containing the solubilized muscle proteins collected. The pH of this phase was then adjusted to pH 5.5 followed by centrifugation at 10,000 x g for 20 min at 4°C to sediment the protein isolate (PI). A conventional surimi process was employed by washing ground fish muscle with 3 volumes of cold water (4°C) three times followed by dewatering in cheesecloth. Surimi and PI's were analyzed for proximate composition and protein recoveries and lipid reduction calculated. Protein isolates were also characterized for their protein composition by SDS-PAGE using pre-cast 4-20% gradient gels (Kelleher and Hultin, 2000). The PI and surimi were held at 4°C and -30°C and lipid oxidation followed by analyzing thiobarbituric acid reactive substances (Lemon, 1975) along with changes in color (L, a, b values, whiteness and hue) using a digital color machine vision system (Luzuriaga, 1999) and a Minolta hand-held colorimeter (Minolta Ltd., Osaka, Japan). Gelation properties of the surimi and PI were tested. For the gelation tests the PI was mixed with 5% sorbitol, 4% sucrose and 0.3% sodium tripolyphosphate and frozen at -30°C. Gel formation (12% protein, pH 7, 500 mM NaCl) on heating (5-80°C) and cooling (80-5°C) was studied in an AR2000 advanced research rheometer (TA Instrument, New Castle, DE) operating in oscillation mode. Conventional cylindrical gels were also made (~20% protein, pH 7, 500 mM NaCl; 80°C for 20 min) using sausage casings and their quality assessed.

Results and Discussion

All species showed highest protein solubility and greatest drop in viscosity in the pH range 1.5-3 and 10.5-12. For this reason pH 2.5 and pH 11 were selected as the processing pH's. Protein recoveries were in general highest when the acid-aided process was used followed by the alkaline process with surimi processing giving the lowest protein yield. Variations in protein recoveries between different species was in part due to variation in their solubility at low and high pH. Taking Spanish mackerel as an example, protein recoveries were as follows: Acid-process; 73.6% > Alkali-process; 69.3% > Surimi; 54.1%. The higher recovery of protein with the acid and alkaline-aided processes is likely due to significantly higher recovery of sarcoplasmic proteins which are largely washed out in surimi processing (Kelleher and Hultin, 2000). The lower recovery of the alkali-aided process was likely due to favorable interactions of the muscle proteins with lipids, creating an emulsion (Kristinsson, 2002). To increase yield even more in the acid- and alkali-aided process the first centrifugation step was omitted, which gave between ~80-90% protein recovery, a practice that can be recommended for oxidatively stable raw materials. Reduction in lipids was in all cases the highest using the alkali-aided process, followed by the acid aided process and with surimi processing giving least reduction. The significantly higher reduction in lipids for both acid and alkali-aided processes is due to the selective separation of the muscle proteins from neutral and polar lipids (membrane phospholipids) during the first centrifugation (Kelleher and Hultin, 2000).

The alkali process gave PI's with good gelling properties while the acid process gave PI's of very poor gel forming ability, except for Atlantic croaker which had a gel strength similar to surimi. Protein electrophoresis did indicate hydrolysis at acid pH may play a role in the poor gelation for the acid PI's. Surimi processing gave proteins with intermediate gel forming ability compared to the acid and alkali produced PI's. The improved gelling ability of the alkali produced PI's could stem from a modification in muscle protein structure yielding enhanced protein-protein interactions (Kristinsson, 2002). Alkali processing in general gave a protein product of better color characteristics (e.g. higher whiteness values) than acid processing. This was found to be due to both more removal of heme proteins during alkali processing and also due to the prevention of heme protein denaturation at alkali processing pH's. Heme proteins were on the other hand substantially denatured at low pH. This effect on heme proteins also gave alkali produced PI's a significantly better oxidative stability on storage for all species tested compared to acid produced PI's. Increased oxidative stability of the alkali produced PI's was also likely due to more lipid removal. Proteins recovered with surimi processing had less oxidative stability than alkali processed PI's, possibly due to higher lipid and heme protein content. Oxidative and color stabilities were found to be significantly different for different species.

Conclusion

Of all three processes studied it was clear that even though the alkali-aided process gave less protein recovery than the acid process it produced protein products of significantly better functional properties and oxidative stabilities than both acid-aided processing and conventional surimi processing. These results demonstrate that the alkali-aided process can be successfully used on warm water fish species to produce protein ingredients of good function and stability, even more so than conventional surimi processing. This study also highlights how different species can respond differently to the two new processes, which underlines the need to properly investigate each species for the successful production of protein ingredients.

Acknowledgements

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P 54. USE OF THE EQUIVALENT POINT METHOD TO OPTIMIZE MICROWAVE COOKING OF SURIMI SEAFOOD PRODUCTS

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Abstract

We previously reported at last year's AFTC the ability to rapidly heat muscle protein sols to produce gels equivalent in textural properties to those conventionally heated more slowly. The equivalent point method (EPM) was lately applied to these data, revealing a possible 'window' of operating conditions wherein acceptable gel properties and cook yields could be obtained. We report here of our progress in applying this approach to rapid microwave cooking of surimi seafoods, which entails gelatinization of added starches as a complicating factor.

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P 55. BIOCHEMICAL AND FUNCTIONAL PROPERTIES OF HYDROLYSATES FROM HERRING *(CLUPEA HARENGUS)* BYPRODUCTS

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Abstract

The use of proteolytic enzymes to hydrolyze fish proteins can alter the functional properties of endogenous proteins while keeping the nutritive value relatively constant. The objective of this study was to characterize the functional, nutritional and the antioxidant properties of fish protein hydrolysates derived from herring and herring byproducts. Whole herring or herring parts (whole body, head, and gonad) ~~parts~~ were hydrolyzed at 50° C for 60 minutes using Alcalase (Novozyme). Aliquots were taken at 0, 15, 30, 45, 60, and 75 minutes to measure degree of hydrolysis (%DH). The hydrolyzed samples were freeze dried and then analyzed for proximate composition, minerals content, amino acids content, functional properties and antioxidant capacity. There were 3 experimental replications (lot of herring) and 3 batches of FPH were made from each replication. All freeze-dried herring hydrolysate (FPH) powders contained 77 to 87% protein. Herring gonad hydrolysates powder was darkest in color (L=74.59) while whole herring hydrolysates protein powder was lightness (L= 89.4). The degree of hydrolysis was 18%, 13%, 13%, and 10% for head, whole fish, fillet, and gonad, respectively. The fish hydrolysates had desirable essential amino acid profiles and mineral contents. The emulsification capacity and emulsification stability of the fish hydrolysates were lower than egg albumin or soy protein. Fat adsorption of fish hydrolysates was comparable to egg albumin. Antioxidant activity of whole herring FPH was highest (48.75%), followed by that of whole body (44. 35%), gonad (33.68%), and head (15.7%) FPHs. Antioxidant activity of whole herring FPHs was about half that of tocopherol (85%), BHA (95%), and BHT (97%). This study indicates that fish protein hydrolysates, derived from herring and herring byproducts under these conditions have commercially desirable functional, nutritional, and antioxidant properties.

Introduction

Proteins from fish processing byproducts can be modified to improve their quality and functional characteristics by enzymatic hydrolysis (Shahidi, 1994). Utilizing proteolytic enzymes, fish protein hydrolysates can be prepared with the peptides having new and/or improved properties. The objective of this study was to develop and characterize FPHs from whole herring, herring body**,** herring head, and herring gonad for use as functional ingredients and nutritional supplements.

Materials and Methods

Whole herring (WH), herring body (HB) (only head and gonads were removed), heads (HH), and gonads (HG) were minced in a Hobart mincer (K5SS, Hobart Corporation, Troy, OH) and hydrolyzed by alcalase enzyme with the activity of 2.4 Anson Units per gram (AU/g) as described (Hoyle and Merritt, 1994). The hydrolysate was centrifuged at 2,560 x *g* for 15 min and the soluble aqueous fraction decanted, freeze-dried, sealed in vacuum bags, and stored at 4°C until used. A separate experiment was performed to determine the degree of hydrolysis (DH) using the method of Hoyle and Merritt (1994). DH was calculated as: DH = [10% TCA - soluble N in the Sample/ Total N in the Sample] x 100.

FPHs were analyzed for biochemical and functional properties. Amino acid analysis were conducted at the AAA Service Laboratory Inc., OR. The mineral content of all FPHs was determined in triplicate by the acid digestion method involving microwave technology (CEM microwave, MDS-2000, CEM Corporation, Matthews, NC) as described by Sathivel and others (2003). Color of the FPH powders was determined using a Minolta color (Minolta Corporation, Ramsey, NJ) and reported as L*, a*, and b*. The SDS-PAGE gel electrophoreses were performed in a Mini-Protein electrophoresis unit (Bio-Rad Laboratories, Richmond, CA) under reducing conditions according to Laemmli (1970).

Purified egg albumin and soy protein concentrate powder (SPC) were used as protein references to compare the functional properties of FPHs. Nitrogen solubility was determined by the procedure of Morr and others (1985). The solubility of FHPs is defined as soluble nitrogen divided by total nitrogen**.** Emulsifying capacity (EC) was measured by an oil titration method similar to that of Webb and others (1970). EC was expressed as milliliters of emulsified oil per 200 mg of protein. Emulsion stability (%) was calculated as [(total volume – aqueous volume)/ total volume] x 100. The antioxidative activity of the FPH was determined by the thiocyanate method (Shih and others, 2002). Food grade antioxidants, α-tocopherol, butylated hydroxyanisole (BHA), and butylated hydroxytoluene (BHT) (Sigma Chemical Co., St. Louis, MO) were used as references. Antioxidative capacity is the capacity to inhibit peroxide formation in linoleic acid was expressed as: Inhibition % = [1-{(absorbance of sample at 500 nm)/(absorbance of control at 500 nm)}] x 100

Results and Discussion

The whole herring (WHH), herring body (HBH) and herring heads (HHH) hydrolysates had high protein contents ranging from 84.4 to 87% while herring gonads (HGH) had a significantly lower value of 77%. The ash content of WHH, HBH and HHH was approximately 10% compared 15% of HGH which was higher. The DH% after 75 min of hydrolysis was 18.3%, 13%, 13%, and 10.1%, respectively, for HH, WH, HB, and HG. The shape of the hydrolysis curve is similar to those previously published for herring protein hydrolysates (Hoyle and Merritt 1994). HGH was darkest with L* value of 74.59 and most yellow (b* = 18), while WHH was the lightest (L* = 89.4) and least yellow (b* = 8.0). All of the herring protein hydrolysates met or exceeded the essential amino acid requirements for adult humans. Potassium, magnesium, phosphorus, sodium, sulfur, and calcium were abundant mineral elements in the FPHs. WHH, HBH, and HHH had above 78% solubility**,** while HGH had about 56% solubility. The solubility of egg albumin was not significantly different than that of WHH and HHH. Soy protein concentrate (SPC) was less soluble than the FPHs, having solubility of 9.8.—–FPHs had lower emulsifying capacities than both of the reference proteins. Emulsifying stability of FPHs ranged from 48.6 to 54.2**%**, which were lower than egg albumin (72.3%) and SPC (62.2%). The difference in emulsifying properties observed among hydrolysates may have been due to hydrophobicity (Gauthier and others 1993) and different peptide lengths. Smaller peptides often have reduced emulsifying properties (Jost and others 1977). FPHs had a protein and peptide molecular weights below 14,400 daltons. WHH had the highest fat absorption capacity (7.3 mL/g protein), whereas SPC had the lowest fat absorption capacity (3.6 mL/g of protein). Both WHH and HHH had a greater ability to bind soybean oil than did HGH, egg albumin or SPC. Degree of hydrolysis appears to affect the ability of hydrolysates to bind fat (Kristinsson and Rasco, 2000). Among herring FPHs, WHH (48.75%) and HBH (44.35%) had much higher antioxidant activities than HHH (15.7%). The antioxidant activity of WHH and HBH was about 0.5 time less than that of α-tocopherol (85%), BHA (95%), and BHT (97%).

Conclusion

The herring FPHs have desirable color, nutritional, antioxidant properties, solubility, fat absorption and emulsification stability. They can potentially compete with hydrolysates and protein powders currently available in the market place.

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P 56. VALORISATION OF UNDER-UTILISED FISH SPECIES FROM THE CANTABRIAN SEA BY MEANS OF SURIMI AND DERIVED SEAFOOD PRODUCTS PRODUCTION

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Abstract

Mackerel (*Scomber scombrus*), horse mackerel (*Trachurus trachurus*) and sardine (*Sardine pilchardus*) are three relatively abundant species in the Cantabrian Sea, but of low commercial interest (low sale price, quick deterioration, small size, etc.). The application of the newest texturization technologies (enzymatic systems, physical methods, extrusion-coextrusion processes and re-structuration using food ingredients and additives) allow the valorisation of these fish species. These technologies not only permit the extension of the commercial life of the fish muscle (through flesh washing or the inclusion of ingredients or preservatives) but also the control of portion sizes, the development of new products with different textures and the use of muscular parts that would otherwise be rejected, with the consequent reduction in the fish wastes. In this work, the surimi production processes from the mentioned species at laboratory and pilot scale have been defined, together with the development of four fish product prototypes targeted to young people and children and the aged population.

Introduction

The production of fish protein concentrates (surimi) by means of physical methods (washing, drying, refining and decanting) is generally a texturization process that uses white fish species as raw materials. It is although susceptible to be applied for the valorisation of fatty fish species (mackerel, sardine and horse mackerel) (Garcia , I and Pozo, R.; 1997 ; Garcia, I. and Pérez-Villarreal, 1998).

The protein concentrates obtained, can be used to develop a large variety of seafood products which satisfy nowadays consumer expectations. The present study describes not only the surimi production process from the mentioned species but also the trials developed to obtain four seafood products prototypes.

Materials and Methods

Raw materials

Mackerel (*Scomber scombrus*), sardine (*Sardina pilchardus*) and horse mackerel (*Trachurus trachurus*) frozen fillets were purchased from a local fish freezing plant. The fish captured in the Biscay Gulf (VIIIb zone) had been stored (frozen in brine) in the plant for (6 months) at -20ºC in 22 kg carton boxes. Cellulose and collagen casings (10-20-25mm ∅) were obtained from Viscofan S.A. (Navarra, Spain). Food ingredients, additives, spices and flavours were supplied by local food ingredients distributors. These included; wheat, maize, potato and tapioca starch, wheat flour, salt, sugar, wheat fiber, cellulose, sunflower oil, xanthan and guar gum, carragenan, alginate, milk, egg and soy proteins, bread, flavours (fishy, meaty, herbal), spices, colorings and preservatives.

Preparation of surimi bases

Mackerel, sardine and horse mackerel surimi were elaborated at laboratory scale. Thawed fish (15ºC, 15 hours) was head-off, eviscerated and filleted by hand and the flesh passed through a mechanical separator (Baader) to separate bones. Then the mince was washed twice in an stainless steel tank adjusting the pH with $NaHCO_3$ (3%). By means of a mechanical press the solid fraction was separated and the mixture mixed with cryoprotectants. Finally the mix was frozen (-35ºC, 1 h 30min.).

In order to see the different characteristics (yield, quality) of the surimi obtained at lab versus industrial scale, mackerel surimi was also produced with a semi-industrial automated surimi line (Protial-France) with a yield 50-60kg surimi/hour. For surimi preparation, the fillets were thawed at 8ºC overnight and after peeling with a skinning-off machine (Varlet), the bone fragments were separated in a mechanical separator (Sepamatic equipment. The mince was washed at 8ºC with running water modifying the pH (optimun 6,5-7) of the mixture with sodium bicarbonate (0,15%). The washed mince was dispatched through a pump (Guerin) to the spin-dryer where the blood proteins and sarcoplasmis proteins are thrown out. The mince was washed again, the pH adjusted and finally was transferred to the refiner (Auriol) where the collagen, bones, scales or small fragments were separated. The product obtained was dispatched to a three way centrifugal decanter (Westfalia Separator CA220-030) to obtain the protein fraction. Cryoprotecting agents were added and finally the mix was molded in trays and frozen in a Stal plate-freezer at –30ºC.

Preparation of surimi based seafood products (sausages and hamburgers)

Re-structured surimi products were prepared using as main raw materials mackerel, sardine and horse mackerel surimi. The final products designed were frankfurter sausages of small size (cocktail size) and small hamburgers (nuggets) with good sensory (texture, appearance, flavour) and technological (industrial processing requirements) attributes. An experimental matrix was designed including the raw materials, ingredients and additives utilized. Up to 50 batches of sausages and hamburgers have been elaborated according to the following general processes (fig 1).

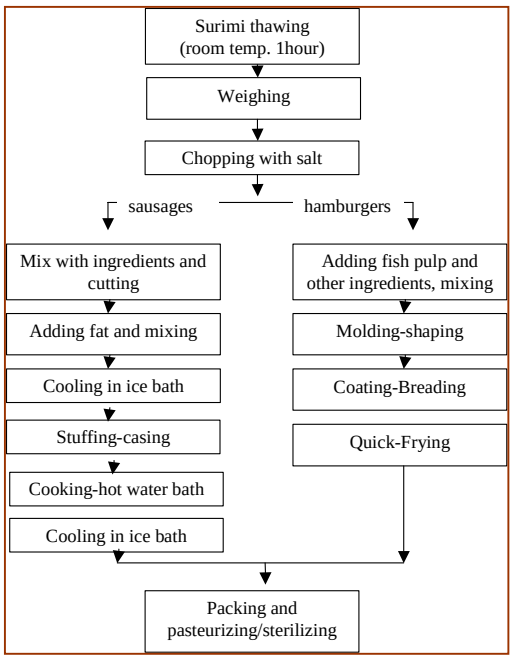
Surimi sausages

Mackerel, sardine or horse-mackerel surimi was thawed at room temperature for 1 hour and then weighed as well as all the other ingredients included in the formulation. The surimi was chopped in a vacuum cutter (Robot-Coupe R 10 V.V.) to avoid the oxidation and presence of air bubbles, for 1-2 min at low speed maintaining the temperature below 8ºC and after, salt and crushed ice were are added too. All the ingredients (depending on the formulation considered) except oil (added last) were mixed and the mixture remained in the bowl until a smooth paste was achieved. The mixture was then placed in a plastic tray and stuffed (sausage stuffing machine – Importadora Tudelana (Spain)) into 10-15-20mm Ø cellulose or collagen casings. Links 5cm length were made. Stuffed sausage products were cooked in a water bath (Gallenkamp) at 90-92ºC for 30 min and quickly cooled in an ice bath. Then the sausages were packed in a vacuum type bag sealing machine (Tecnotrip). The samples were stored at 7-8ºC until being evaluated.

Surimi hamburgers

After chopping the surimi (mackerel, sardine, horse mackerel) with salt in the cutter different proportions of these fatty fishes pulp were added as well as the rest of the ingredients. When a coarse grinding was achieved the mixture was molded in a hamburger making machine (Mainca) and enrobed in a bread covering. After a quick frying in vegetable oil the products were vacuum packed (Tecnotrip) and stored at 7-8ºC until being evaluated.

Fig.1: Surimi sausages and hamburgers manufacturing process



Results and Discussion

Surimi seafood products (sausages and hamburgers)

Although more than 50 formulations of surimi sausages and hamburgers were developed, 4 prototypes of these products (2 cocktail sausages (CS) and 2 small hamburgers (SH)) were selected according to its sensory characteristics, (texture, appearance, flavour) when consumed cold, fried or after microwave heating and technological (industrial processing requirements) attributes. These prototypes were targeted to young people, children and aged population.

In the formulations different proportions of mackerel, sardine and horse mackerel surimi as well as other ingredients were included in order to obtain the desired texture (farkfurter sausage texture), appearance and flavour.

Formulations

CS1- Cocktail sausages in collagen casings 20mm Ø and 5 cm long. Mackerel surimi (59,50%), crushed ice (22,5%), salt (2%), potato flour (6%), guar gum (1%), soy protein (3%), wheat fiber (2%), sunflower oil (3%), chicken flavour (0,85%) , salmon coloring (0,15%).

SH1-Small hamburgers (nuggets), circular shaped 5 cm Ø. Horse mackerel surimi, (25%) egg white (3%), onion (2%) horse mackerel pulp (56%), crushed ice (12%), milk whey (2%), covering (bread , binding agent).

Conclusions

Some texturation technologies such as surimi processing can be helpful tools to valorise under-utilised fish species and obtain new fish products suitable for specific groups of population.

At lab scale the final yield of the processes (surimi base /whole fish) for the three species considered, was about 23 %, depending on fish size and process conditions.

Mackerel surimi obtained at lab and semi-industrial scale showed significant different quality properties. In spite of their low gellifying power, the fish protein concentrates elaborated from fatty fish species such as mackerel, sardine and horse mackerel, could be incorporated in the formulations of these new products and satisfy the consumers expectations with respect to texture, flavour and modern cooking habits.

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P 57. FUNCTIONAL PROPERTIES OF DIFFERENT FRACTIONS GENERATED FROM HYDROLYSED COD (*GADUS MORHUA)* BY-PRODUCTS

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Abstract

Annually more than 91 million tons of fish and shellfish are harvested worldwide. Referring to a survey done by Rubin, about 54 % of the by-products of Norwegian cod catch was discarded in the year 2001 (www.rubin.no). Approximately 30% of the total catch is used for fish meal and animal feed because of its poor functional properties. Hence, optimal utilisation of fishery by-products is becoming increasingly important to provide more marine fish raw material for various purposes. One of the approaches for effective protein recovery from by-products is enzymatic hydrolysis, which can be applied to improve and upgrade the functional and nutritional properties of proteins.

The aim of this study was to evaluate the relationship between the chemical composition of by-products from cod (*Gadus morhua)* and the yield and functional properties of different fractions such as of oil, fish protein hydrolysate and non-soluble precipitates. Different by-products were treated with commercial proteases and the effects of (1) the type of enzyme and of (2) the addition of water were evaluated. Differences in the quantitative and qualitative distribution of lipid classes were evaluated in the different fractions. Functional properties such as degree of hydrolysis, water holding capacity, fat absorption of protein contained factors were also measured. Lipid oxidation was evaluated by traditional methods like the peroxide value and thiobarbiture reactive substances (TBARS) together with Electron Spin Resonance (ESR) spectroscopy to study the free radicals formed.

To achieve better utilisation of all fish by-products it is necessary to pay more attention to the sludge, part that is non-soluble in water after hydrolysis. Yield of the sludge was significantly higher ($p<0.05$) compared to FPH yield. The sludge contained a high amount of oil (up to 33.4 g/100g) including high concentration of phospholipids (up to 54.5 g/100g). The phospholipids do not seem to be easily extracted from fish by-products by water/enzyme extraction. This may be a very good starting material for subsequent extraction of phospholipids. Therefore with a view to find better utilisation of the sludge more description of the physiochemical and functional properties should be done.

It is evident, that it is not possible during centrifugation to separate all the oil. First of all the aim of protein hydrolysis is to obtain hydrolysates with the lowest amount of oil. Second, heat inactivation of enzymes and raised temperatures reduce quality of oil by increasing the amount of FFA. Also the denatured proteins seem to be highly resistant to enzymatic breakdown and probably form protein – lipid complexes, which may also reduce extraction of oil. Simultaneously, formation of aggregates reduces extraction of oil and yield of FPH fraction. So, more attention should be taken to the temperature regime during inactivation and hydrolysis, which can give higher yields and better quality both of the oil and protein containing fractions.

Based on amino acid composition both FPH and sludge may serve as a potential nutrient component. Results showed that the most important factor for the yield of different fractions, physiochemical and functional properties of oil, FPH and sludge was amount of added water rather than type of enzymes used. The highest oil yield was obtained in the samples without addition of water. Elimination of extra water increased amount of sludge and decreased FPH yield. By reducing amount of added water a reduction in amount of lipids in the sludge and FPH was also observed. The optimal raw material - added water ratio should be defined for the processes considering the desirable properties of the end products.

FPH and sludge obtained without adding water showed slightly higher fat absorption capacity. The most significant factor influencing emulsification capacity of FPH was amount of water added.

The lipid oxidation proceeded faster in the oil that was separated after enzymatic treatment compared to the oil that was chemically extracted. These differences were not noticeable when analysing the oxidation level without further storage. This might be due to the fact that more lipid components (including antioxidative components) are extracted chemically and mainly triacylglycerols are separated mechanically.

The oxidation level was, as expected, higher in the dried FPH and much higher in the sludge (peroxide values up to 350 mEq/kg) compared to the oil. The free radicals formed in the different phases where not correlated to the results from the other analytical methods. This might be due to a formation of other radicals than the ones formed during lipid oxidation.

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11-14 June 2003, Reykjavik - Iceland

K 6. MARINE OILS AND BIOACTIVE COMPOUNDS AS NUTRACEUTICALS AND FUNCTIONAL FOOD INGREDIENTS: CURRENT STATUS AND FUTURE TRENDS

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Abstract

Marine nutraceuticals encompass a wide array of products such as fish oils, seal oil, fish liver oils, algal products, bioactive peptides and protein hydrolyzates, chitosan and its products, among others. These are often used in the medicinal form, as supplements or as functional food ingredients. As an example, highly unsaturated fatty acids derived from marine oils exhibit important biological activity. They also serve as building block fatty acids in the brain, retina and other organs with electrical activity. Hence their inclusion in the diet, especially for lactating women and infants is encouraged. In addition, protein hydrolyzates and chitinous materials serve important functions in immune enhancement/stimulation and other beneficial effects. This overview provides an account of important issues about the chemistry, quality, health benefits and application of marine nutraceuticals.

Marine Oils

The beneficial health effects of consumption of marine foods, in general, and of marine oils, in particular, in reducing the incidences of coronary heart disease (CHD) are well recognized. The omega-3 fatty acid constituents of marine oils and seafods are considered to be responsible for such attributes. In general, three stages are contemplated in the occurrence of heart attacks in humans. These include, i) injury to the coronary walls; ii) narrowing of the artery due to the build up of a fibrous plaque, known as atherosclerosis; and iii) formation of a blood clot, known as thrombosis. Thus, if a blood clot is formed in an artery which is injured and narrowed down by atherosclerosis, a heart attack may occur. Omega-3 fatty acids are known to reduce the incidence of CHD by lowering the level of serum triacylglycerols and in some cases cholesterol and possibly lower the blood pressure and decrease the venticular arthyrmias, among others. In addition, omega-3 fatty acids are known to relieve arthritic swelling and possibly pain, type II diabetes and to enhance body immunity (Shahidi and Kim 2002a). However, omega-3 fatty acids may increase fluidity of the blood and hence their consumption by patients on blood thinners such as warfarin and aspirin should be carefully considered in order to avoid any unnecessary complication due to vasodilation and possible rupture of capillaries. The omega-3 fatty acids, especially docosahexaenoic acid (DHA), are known to dominate the fatty acid spectrum of brain and retina lipids and play an essential role in the development of fetus, infants and the health status and body requirements of pregnant and lactating women, among others (Simopoulos 1991; Gibson *et al*. 1994).

Table 1. Major fatty acids of omega-3 rich marine and algal oils

Fatty acid	Seal blubber	Cod liver	Menhaden	Algal (DHASCO)
14:0	3.73	3.33	8.32	14.9
16:0	5.58	11.01	17.4	9.05
16:1ω7	18.0	7.85	11.4	2.20
18:0	0.88	3.89	3.33	0.20
18:1ω9+ω11	26.0	21.2	12.1	18.9
20:1ω9	12.2	10.4	1.44	-
20:5ω3	6.41	11.2	13.2	-
22:1ω11	2.01	9.07	0.12	-
22:5ω3	4.66	1.14	2.40	0.51
22:6ω3	7.58	14.8	10.1	47.4

The long-chain omega-3 fatty acids, similar to their omega-6 counterparts, are formed from corresponding C18 precursors by a series of chain elongation and desaturation. While in a healthy individual, where the enzyme system of the body works

adequately, eicosapentaenoic acid (EPA), docosapentaenoic acid (DPA) and DHA can be formed, their inclusion in the diet especially during infancy and old-age as well as pregnancy and lactation is highly recommended.

The primary sources of long-chain omega-3 fatty acids are mainly phytoplankton and algae which serve as feed for marine organisms such as fish (Kyle 2001). Thus, both algal and marine species may be employed for production of omega-3 lipids. Marine oils originate from the liver of lean white fish such as cod and halibut, body of oily fish such as salmon, herring, mackerel and anchovy or the subcutaneous or blubber of marine mammals such as whale and seal. Following processing of highly unsaturated marine oils, the refined, bleached, deodorized (RBD) and stabilized oils, may be used in different applications (Finley and Shahidi 2001).

Nutraceutical Lipids Containing Omega-3 Fatty Acids

Marine oils. The amount and type of long-chain omega-3 fatty acids in marine sources are varied (Table 1). The highest content of EPA (13.2%) occurs in menhaden oil while seal blubber oil has the lowest (6.41%). Meanwhile, DPA is found at 4.68% in seal blubber oil at 2.40% in menhaden oil.

The three dimensional structures of unsaturated fatty acids shows that bending of the molecules increases with an increase in the number of double bonds in their chemical structure and this is further influenced by the position of the double bonds (i.e. omega-3 versus omega-6). These structural features in the triacylglycerol molecules as well as the location of the fatty acids in the glycerol (i.e. sn-1, sn-2 and sn-3) may have a determining effect on the availability of fatty acids involved and their potential health benefits. Presence of highly unsaturated fatty acids in the sn-2 position generally leads to their better assimilation into the body and also offers a better stability to the products.

Table 2. Distribution of long-chain omega-3 fatty acids in menhaden and seal blubber oils

Fatty acid	Seal blubber			Menhaden		
	sn-1	sn-2	sn-3	sn-2	sn-2	sn-3
EPA	8.36	1.60	11.2	3.12	17.5	16.3
DPA	3.99	0.79	8.21	1.12	3.11	2.31
DHA	10.5	2.27	17.9	4.11	17.2	6.12

Thus, in considering two important sources of omega-3 fatty acids, namely menhaden oil and seal blubber oil, omega-3 fatty acids, especially DHA, are primarily located in the sn-2 position in menhaden oil, but mainly in the sn-1 and sn-3 positions of seals blubber oil (Table 2) (Wanasundara and Shahidi 1997a). These differences, undoubtedly, have a definite influence on their assimilation, absorption and health benefits as well as reactions in which they are involved.

Regardless of the source of long-chain omega-3 fatty acids, such oils must undergo appropriate processing. Thus refining, bleaching, deodorization and addition of appropriate antioxidant stabilizers must be practiced in order to allow use of these oils in food formulations (Finley and Shahidi 2001). The type of food in which such omega-3 oils may be incorporated is listed in Table 3. These include foods that could be used within a short period of time and in which the products do not develop off-flavors during their useful shelf-life.

Omega-3 Concentrates. For therapeutic purposes the natural sources of omega-3 fatty acids, as such, may not provide the necessary amounts of these fatty acids and hence concentration of omega-3 fatty acids may be necessary. The omega-3 fatty acid concentrates may be produced in the free fatty acid, simple alkyl ester and acylglycerol forms (Wanasundara *et al*. 2002). To achieve this, physical, chemical and enzymatic processes may be employed for concentrate production. The available methods suitable for large-scale production include low-temperature crystallization, supercritical extraction, molecular distillation, chromatography, urea complexation and enzymatic splitting.

Among the simplest methods for concentrate production is fractional crystallization which takes advantage of the existing differences in the melting point of different fatty acids, as neat compounds or in different solvent systems (Brown and Kolb 1955; Haraldsson 1984). The more saturated fatty acids have higher melting points and may crystallize out of the mixtures and hence leaving behind, in the liquid form, the more unsaturated fatty acids. Obviously, the free fatty acids and simple alkyl esters are more amenable to provide a higher concentration of omega-3 polyunsaturated fatty acids (PUFA) than acylglycerols. This is because the latter mixtures consist of fatty acids with varying chain length and degree of unsaturation affording SSS, SUS, USS, SSU, USU, UUS, SUU and UUU combinations. Thus, total omega-3 PUFA of seal blubber oil was 30.5% at -60°C in hexane and 43.8% in acetone. For free fatty acids of SBO, the corresponding values were 58.3 and 56.8% (Wanasundara *et al.* 2002).

Fractional distillation (FD) is another facile process for separation of mixtures of fatty acid esters under reduced pressure (Brown and Kolb 1955). However, due to sensitivity of more highly unsaturated fatty acids to oxidation, one may use molecular distillation which does not suffer from

Table 3. Food application of omega-3 oils

Food	Country
Bread/hard bread	Australia, France, Germany, Ireland, Denmark
Cereals, crackers & noodles	France, Korea, Taiwan
Bars	USA
Pasta and cakes	France, UK
Infant formula	Australia, Brazil, Japan, New Zealand, Taiwan, UK
Milk, fortified	Argentina, Indonesia, Italy, Spain, UK
Juices, fortified	Brazil, Germany, Spain
Mayonnaise & salad dressings	Korea
Margarines & spreads	Ireland, Japan, UK
Eggs	USA, UK
Canned tuna steak & seafood	Japan, USA
Tuna burger	USA

such drawbacks. While FD of menhaden oil ethyl ester increased EPA from 15.9 to 28.4% and DHA from 9.0 to 43.9 %, molecular distillation afforded DHA with 90% purity (Max 1989).

Reverse phase chromatography has been used by Nakahara *et al*. (1996) to produce a DHA and DPA concentrate from marine microalgae. Teshima *et al*. (1978) used a silver nitrate-impregnated silica gel column to separate EPA and DHA from squid liver oil fatty acid methyl esters. The yield of the process was 39, and 48%, respectively, with 85-96%, EPA at 95-98% DHA purity. Similar studies on a variety of other oils have recently appeared in the literature using high performance liquid chromatography (Tokiwa *et al*. 1981; Adolf and Emiken 1985; Hayashi and Kishimura 1993; Corley *et al*. 2000). More recently, centrifugal partition chromatography (CPC) has gained attention for production of omega-3 concentrates (Murayama *et al*. 1988; Goffic 1997).

Supercritical fluid extraction (SFE) is a relatively new process which is desirable for separation of PUFA. Since this method is based on separation of compounds based on their molecular weight and not their degree of unsaturation, a prior concentration step may be required in order to concentrate omega-3 PUFA. Thus omega-3 fatty acids have been concentrated by SFE from fish oil and seaweed (Yamagouchi *et al*. 1986; Choi *et al*. 1987; Mishra *et al*. 1993). Fish oil esters were fractionated by SFE to obtain an oil with 60-65% DHA (Stout and Spinelli 1987).

Another possibility which allows concentration of omega-3 fatty acids is urea complexation. The natural acylglycerols are hydrolyzed to their fatty acid constituents in ethanol and the resultant components are allowed to crystallize in the presence of urea. While urea alone crystallizes is a tetragonal form (channel diameter 5.67Å), in the presence of fatty acids the molecules of urea crystallize in a hexagonal pattern (channel diameter 8-12Å) with inclusion of saturated fatty acids (Schlenk 1954), known as urea complexing fraction (UCF), and then monoenes followed by dienes and trienes. The non-linear highly unsaturated fatty acids are not included in the urea crystals and remain in the liquid form, referred to as non-urea complexing fraction (NUCF). In this manner, optimum conditions may be employed for preparation of concentrates. If necessary, the urea complexation process may be repeated in order to enhance the concentration of certain fatty acids in the final products. We have used such techniques to prepare concentrates dominated by DHA, EPA or DPA. The total omega-3 fatty acids in one such preparation from seal blubber oil was 88.2% and this was dominated by DHA (67 %) (Shahidi and Wanasundara 1999). Concentration of gamma-linolenic acid from borage oil was also achieved using urea cmplexation process (Spurvey and Shahidi 2000).

Finally, enzymatic procedures may be used to produce concentrates of omega-3 fatty acids. Depending on the type of enzyme, reaction time, temperature and the concentration of the reactants and enzyme, it is possible to produce concentrates in different forms, e.g. as free fatty acids or as acylglycerols. Thus, processes such as transesterification, acidolysis, alcoholysis and hydrolysis as well as esterification of fatty acids with alcohols or glycerol may be employed.

In addition to long-chain omega-3 fatty acids, other fatty acids of importance might be concentrated or used in combination with long-chain omega-3 fatty acids in order to produce concentrates or to obtain specialty lipids (Wanasundara and Shahidi 1997b). The other fatty acids of nutritional and/or therapeutic importance include medium-chain fatty acids (MCFA), conjugated linoleic acid (CLA) and gamma-linolenic acid (GLA), among others.

Wanasundara and Shahidi (1998) have shown that enzymes might be used to selectively hydrolyze saturated and has unsaturated lipids from triglycerols hence concentrating the omega-3 fatty acids in seal blubber and menhaden oils in the acylglycerol form. In this manner, the omega-3 PUFA content was nearly doubled. Furthermore, following urea completion, omega-3 concentrates obtained may be subjected to esterification with glycerol to produce concentrated acylglycerols. Upon glycerolysis of specialty alkyl esters from seal blubber oil, we found that monoacylglycerols, diacylglycerols and triacylglycerols were formed simultaneously. The amount of monoacylglycerols decreased continuously while that of triacylglycerols increased (He and Shahidi 1997; Wanasundara and Shahidi 1998).

Structured Lipids. Structured lipids (SL) are TAG containing combinations of short-chain fatty acids (SCFA), medium-chain fatty acids (MCFA) and long-chain fatty acids (LCFA) located in the same glycerol molecule and these may be produced by chemical or enzymatic processes (Lee and Akoh 1998; Senanayake and Shahidi 2000). These specialty lipids may be produced via direct esterfication, acidolysis, and hydrolsis or interestification. Structured lipids are developed to fully optimize the benefits of their fatty acid varieties in order to affect metabolic parameters such as immune function, nitrogen balance and lipid clearance from bloodstream.

Various fatty acids are used in the production of structured lipids and these may include both omega-3 and omega-6 fatty acids, among others. Structured lipids containing MCFA and LCFA may modify the absorption rates because MCFA are rapidly oxidized for energy while LCFA are oxidized very slowly. These specialty lipids are structurally and metabolically different from simple physical mixtures of medium-chain and long-chain triacylglycerols.

MCFA are structured fatty acids with 6-12 carbon atoms and are often used for production of SL. As mentioned earlier, MCFA are highly susceptible to β-oxidation (Odle 1997). These fatty acids are not stored in the adipasc tissues and are often used in the diet of patients with maldigestion and malabsorption (Willis *et al*. 1998). They have also been employed in total parenteral nutrition and formulas for preterm infants. In addition, calorie-reduced SL containing MCFA have recently been developed and represent exciting new application areas (Finley *et al*. 1997).

Table 4. Enzymatic modification of seal blubber oil with capric acid using Lypozyme-IM

Fatty acid (%)	Before modification	After modification	sn-2 after modified
10:0	-	27.1	14.9
14:0	3.4	2.7	51.9
14:1	1.0	0.8	41.7
16:0	5.0	3.7	53.2
16:1ω7	15.1	11.9	44.5
18:1ω9 & ω11	26.4	19.3	43.9
18:2ω6	1.3	1.7	33.3
20:ω9	15.0	9.1	27.5
20:5ω3	5.4	2.3	68.1
22:1ω11	3.6	1.9	47.4
22:5ω3	4.9	3.0	23.3
22:6ω3	7.0	7.6	17.9

We have recently reported production of SL via acidalysis of blubber oil with capric acid. Lipozyme-IM from *Mucor miehei* was used as a biocatalyst at an oil to fatty acid ratio of 1:3 in

hexane, at 45°C for 24 h and 1% (w/w) water (Senanayake and Shahidi 2002). Under these conditions, a SL containing 2.3% EPA, 7.6% DHA at 27.1% capric acid (CA) was obtained. While solvents with a log P value of 2.5-4.5 performed well, solvent-free systems also afforded satisfactory incorporation of CA into seal blubber oil. In this product, CA molecules were primarily located in the sn-1 and sn-3 positions, thus releasing them upon the action of pancreatic lipase (see Table 4). Similar results were obtained upon acidolysis of seal blubber with lauric acid (Senanayake and Shahidi 2002). The main portion of capric acid was in positions sn-1 and sn-3, thus serving as a readily available source of energy.

In an effort to produce specialty lipids containing both omega-3 PUFA and GLA, we have prepared such products under optimum conditions (Senanayake and Shahidi 2000). GLA is found in relatively large amounts in borage oil (20-25%), evening primrose oil (8-10%) and blackcurrent (15-18%) oil. Using borage oil, urea complexation process afforded a concentrate with 91% GLA under optimum reaction conditions.

Lipase-catalyzed acidolysis of seal blubber oil (SBO) and menhaden oil (MO) with GLA concentrate, under optimum conditions, that is mole ratio of GLA to TAG of 3:1, reaction temperature of 40°C over 24 h and 500 units enzyme/g oil afforded products with 37.1 and 39.6% GLA incorporation and of the two enzymes tested lipase PS-30 from pseudonomas species served better in the acidolysis protein than *Mucor miehei* (Spurvey *et al*. 2001). Incorporation of GLA was in all positions and its content in sn-2 position of both SBO and MO was 22.1 and 25.7%, respectively (Table 5). Thus, PS-30 served in a non-specific manner in the acidolysis process. The structured lipids containing GLA, EPA and DHA so produced may have potential health benefits.

Production of structured lipids containing GLA, EPA and DHA may also be achieved using borage and evening primrose oils as sources of GLA and either EPA and one of DHA or their combinations (Senanayake and Shahidi 1999a,b). The products so obtained, while similar to those produced by incorporation of GLA into marine oils, differ in the composition and distribution of other fatty acids.

Table 5. Fatty acids of seal blubber oil (SBO), menhaden oil and their acidolysis products with γ-linolenic acid (GLA)

	SBO			MO		
Fatty acid	Unmodified	Modified	sn-2 modified	Unmodified	Modified	sn-2 modified
14:0	3.36	2.40	41.7	8.18	4.55	46.7
16:0	5.14	3.04	48.9	19.89	8.78	46.5
18:1ω9	22.6	14.1	53.4	9.86	4.24	46.3
18:3ω6	0.59	37.1	22.1	0.43	39.6	25.7
20:1ω9	17.3	8.30	44.6	1.62	0.83	80.0
20:5ω3	5.40	3.80	15.4	12.9	11.0	34.1
22:5ω3	5.07	2.99	22.0	2.48	2.07	33.3
22:6ω3	7.73	4.36	20.8	10.0	6.56	22.6

Oxidative Stability. The oxidative stability of modified lipids is affected, to a large extent, by the type of process employed for their modification as well as the type of product obtained. Thus, affect the stability of structured lipids is dictated primarily by the composition of their fatty acids as well as the content and distribution of minor components with antioxidant activity (Shahidi 2002). This incorporation of saturated fatty acids would, in general, may lead to better stability of products while introduction of PUFA may reduce the oxidative stability of the oils. Modified oils, in general, contain a lesser amount of endogenous antioxidants due to their removal during the experimental unit operations. This would contribute to compromised stability of the resultant oils. This situation was recently demonstrated when stability of a structured lipid from acidolysis of an algal oil (DHASCO) with capric acid was compared with that of the original oil. Results indicated that despite its higher degree of saturation, the structured lipid so produced was less stable than the original DHASCO. However, when reactants were subjected to the same process unit operations in the absence of any enzyme, the oil so processed was considerably less stable than its natural counterpart. Therefore, the oils as such or after modification may be stabilized using synthetic or preferably natural antioxidants. Thus, oxidation of menhaden oil at 60°C over a 7 day period was inhibited by 54.7% by TBHQ (200 ppm), 32.5% by mixed tocopherols (500 ppm), 18.0% by α-tocopherol (500 ppm), 39.8% by mixed green tea catechins (200 ppm) 45.1% by EC; 48.2% by EGC; 51.3% by ECG and 50.0% by EGCG, all at 200 ppm (Shahidi and Kim 2002b). For seal blubber oil, the best protection was provided by TBHQ (200 ppm) by 56.3% inhibition and ECG (200 ppm) at 58.6%. Alpha-tocopherol inhibited oxidation by only 14.2% (Shahidi and Kim 2002).

Table 6. Antioxidative peptides from gelatin hydrolyzate of Alaska Pollack skin in comparison with that of soy 75 protein.

Peptide	Amino acid sequence
	ALASKA POLLACK SKIN
P_1	Gly-Glu-Hyp-Gly-Pro-Hyp-Gly-Pro-Hyp-Gly-Pro-Hyp-Gly-Pro-Hyp-Gly
P_2	Gly-Pro-Hyp-Gly-Pro-Hyp-Gly-Pro-Hyp-Gly-Pro-Hyp-Gly
	Soy 75 PROTEIN
P_1	Val-Asn-Pro-His-Asp-His-Glu-Asn
P_2	Leu-Val-Asn-Pro-His-Asp-His-Glu-Asn
P_3	Leu-Leu-Pro-His-His
P_4	Leu-Leu-Pro-His-His-Ala-Asp-Ala-Asp-Tyr
P_5	Val-Ile-Pro-Ala-Gly-Tyr-Pro
P_6	Leu-Glu-Ser-Gly-Asp-Ala-Leu-Arg-Val-Pro-Ser-Gly-Thr-Tyr-Tyr

Bioactive Peptides from Marine Resources

Cleavage of amide linkage in the protein chain leads to the formation of peptides with different number of amino acids as well as free amino acids. While enzymes with endopeptidase activity provide peptides with different chain length, exopeptidases liberate amino acids from the terminal positions of the protein molecules. Depending on reaction variables as well as the type of enzyme, the degree of hydrolysis of proteins may differ considerably. The peptides produced from the action of a specific

enzyme may be subjected to further hydrolysis by other enzymes. Thus, an enzyme mixture or several enzymes in a sequential manner may be used. The peptides so obtained may be subjected to chromatographic separation and then evaluated for their amino acid sequence as well as their antioxidant and other activities.

In a study on capelin protein hydrolyzates, four peptide fractions were separated using Sephadex G-10. While one fraction exerted a strong antioxidant activity in a β-carotene-linoleate model system, two fractions possessed a weak antioxidant activity and the fourth had a prooxidant effect. Two dimentional HPTLC separation showed spots with both pro- and antioxidantive effects (Amarowicz and Shahidi, 1997). Meanwhile, protein hydrolyzates prepared from seal meat were found to serve as phosphate alternatives in processed meats and reduced the cooking loss considerably (Shahidi and Synowiecki, 1997). Furthermore, Alaska pollack skin hydrolyzate was prepared using a multienzyme system in a sequential manner. The enzymes used were in the order of Alcalase, Pronase E and collagenase. The fraction from the second step, which was hydrolyzed by Pronase E, was composed of peptides ranging from 1.5 to 4.5 kDa and showed a high antioxidant activity. Two peptides were isolated, using a combination of chromatographic procedures, and these were composed of 13 and 16 amino acid residues (Kim *et al*., 2001). The sequence of the peptides involved is given in Table 6 and compared with those of soy 75 protein hydrolyzates (Chen *et al*., 1995). These peptides exert their antioxidant activity via both free radical scavenging as well as chelation effects. Recently, proteases from shrimp processing discards were characterized (Kim *et al*., 2003) and application of salt-fermented shrimp by-product sauce (Unpublished results) were used as a meat tenderizer.

Table 7. Some applications of chitosan and its products.

Product	Effect
Chitosan	Weight loss, hypocholesteroleuic, control release of nutrients and drugs, inhibitor of oxidation, etc.
Chitosan oligomers	Anti-inflammatory, antimicrobial, etc.
N-acetylglucosamine	Anti-inflammatory, etc.
Glucosamine	Joint built, anti-inflammatory, etc.

Chitin, Chitosan and Related Compounds

Chitin is recovered from processing discards of shrimp, crab, lobster and crayfish following deproteinization and demineralization (Shahidi and Synowiecki, 1991; Shahidi *et al*., 1999). The chitin so obtained may then be deacetylated to afford chitosan (Shahidi and Synowiecki, 1991). Depending on the duration of the deacetylation process, the chitosan produced had different viscosities and molecular weights. The chitosans produced are soluble in weak acid solutions, thus chitosan ascorbate, chitosan acetate, chitosan lactate and chitosan malate, among others, may result and these are all soluble in water. Chitosan has a variety of health benefits and may be employed in a number of nutraceutical and health-related applications (Table 7). Chitosan derivatives may also be produced in order to obtain more effective products for certain applications. However, to have the products solubilized in water without the use of acids, enzymatic processes may be carried out to produce chitosan oligomers. Due to their solubility in water, chitosan oligomers serve best in rendering their benefits under normal physiological conditions and in foods with neutral pH. Furthermore, depending on the type of enzyme employed, chitosan oligomers with specific chain length may be produced for certain applications (Jeon *et al*., 2000).

Chitosans with different viscosities were prepared (Table 6) and used in an experiment designed to protect both raw and cooked fish against oxidation as well as microbial spoilage (Jeon *et al*., 2002; Kamil *et al*., 2002; Shahidi *et al*., 2002). The content of propanal, an indicator of lipid oxidation, was decreased when chitosan was used as an edible invisible film in herring. Furthermore, the effects were more pronounced as the molecule weight of the chitosan increased (Table 7). In addition, inhibitory effects of chitosan coatings in the total microbial counts for cod and herring showed an approximately 1.5 and 2.0 log cycles difference between coated and uncoated samples, respectively, after 10 days of refrigerated storage (results not shown). The monomer of chitin, N-acetylglucosamine (NAG) has been shown to possess anti-inflammatory properties. Meanwhile, glucosamine, the monomer of chitosan, prepared via HCl hydrolysis, is marketed as glucosamine sulfate. This formulation is prepared by addition of ferrous sulfate to the preparation. Glucosamine products may also be sold in formulation containing chondroitin 4- and 6-sulfates. While glucosamine helps to form proteoglycans that sit within the space in the cartiledge, chondroitin sulfate acts like a liquid magnet. Thus glucosamine and chondroitin work in a complementary manner to improve the health of the joint cartiledge.

The by-products in chitin extraction process from shellfish include carotenoids/carotenoproteins and enzymes (Shahidi, 1995; Shahidi *et al*., 1998; Shahidi and Kamil, 2001). These components may also be isolated for further utilization in a variety of applications.

Future Trends

Exponential increase in health care and hospital bills is serving as a catalyst in efforts for disease prevention and health promotion through dietary means. Thus, future efforts should be directed in meeting the DRI (daily required intake) of dietary components such as omega-3 fatty acids and to replace epidemiological and anecdotal points of reference with appropriate clinical, hopefully using double-blind, studies. Quality control must also be strictly followed in order to offer the best products with good sensory attributes of taste and flavor of the consumers.

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L 64. DEEP-SEA SPECIES – A SOURCE FOR HUMAN CONSUMPTION AND NOVEL COMPOUNDS WITH BENEFICIAL HEALTH EFFECTS.

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Abstract

Deep-sea species are affected by large pressure, absence of light and relatively low supply of food. Sharks, including deep-sea sharks, are known to be among the healthiest animals alive. From literature it is known that shark liver oil show antibacterial, antioxidative, anticarcinogenic, immune stimulating and antiobesity effects. Chemical composition of muscle, head, brain, liver and eggs has been studied for the shark species *Centrocymnus coelolepis*, *Centrophorus squamosus*, and *Centrocyllium fabricii*. The oil fractions contained high amounts of unsaturated fatty acids. Liver fractions contained from 11 % to 84 % squalene, and showed antibacterial activity.

Introduction

There is an increased commercial interest for deep-sea species, but many species are still underutilized. Our main focus is to improve the utilization of these species and find bioactive compounds of commercial interest.

The objective of our study is to examine important chemical components in deep-sea sharks, in both flesh and byproducts. Regarding deep-sea sharks, most interest has been on fillets and liver oil. Recently there has also been an increased interest to examine components in other organs. These organs may be rich in polyunsaturated fatty acids and other biological active components.

Fish heads are a good source for proteins and minerals. Lipids from brains have a fatty acid composition rich in polyunsaturated fatty acids (PUFAs), which may be used for production of nutraceuticals and functional food.

The species studied have a viviparous (live bearing) reproduction. Eggs in the uterus are about 2-10 cm in diameter. The eggs contain all the nutrients required by the developing embryo up to the time of hatching, and could be a possible source of bioactive compounds with commercial value.

The oil derived from deep-sea shark livers usually contain high levels of squalene, diacylglyceryl ethers and triacylglycerols and minor levels of free fatty acids, sterol, pristine, wax esters and sterol esters (Oh 2001, Peyronel and others 1984, Bakes and Nichols 1995). Previous studies have shown that squalene and alcylglycerol in the liver are responsible for the sharks amazing ability to resist cancer. It has also been proved that squalene is a strong antioxidant, and that it has an anti-tumor activity and immunostimulatory effect.

Squalamine is an aminosterol with antibiotic activity, discovered in tissue extracts of *Squalus acanthias* (spiny dogfish shark) (Moore and others 1993). The liver also contains smaller amounts of structurally related aminosterols, each exhibiting broad spectrum activity against Gram-negative and Gram-positive bacteria and fungi (Rao and others 2000). These aminosterols have remarkable properties, including inhibition of angiogenesis, appetite suppression, and inhibition of sodium proton exchangers (Sills and others 1998, Williams and others 2001).

Materials and methods

The deep-sea sharks *Centrocymnus coelolepis*, *Centrophorus squamosus*, and *Centrocyllium fabricii* were captured on long lines at Hatton Bank in May-June, 2000, at depth around 800-1500 meters. The sharks were immediately killed. The byproducts were sorted and kept frozen at -30 °C, until processing.

The content of protein was measured according to the directive 93/28/EEC (Official Journal of the European Communities). The lipids were extracted according to method by Bligh and Dyer. The fatty acid composition was measured according to AOCS Official Method Ce 1b-89. Antimicrobial activity against *Escherichia coli (*ATTC 25922), *Staphylococcus aureus* (ATTC 29213) and *Candida albicans* (ATTC 90028), was analyzed by measuring clearing zone on a microbial lawn in Petri plates.

Results and discussion

The chemical composition of muscle, head and eggs has been studied, and results are shown in Figure 1. The factor 5,4 (Oehlenschläger 1992) has been used to calculate the protein content in the shark species, because of the known high content of non-protein nitrogen.

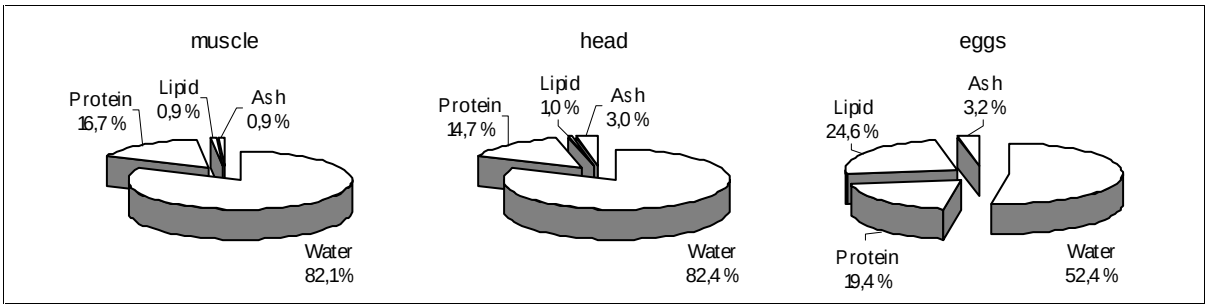

Figure 1: The chemical composition (% content of the wet weight) of muscle, head and eggs from *C. coelolepis*, *C. squamosus* and *C. fabricii*.

The fatty acid composition of fat extracted from muscle, brain and eggs from *C. coelolepis*, *C. squamosus*, and *C. fabricii* is shown in Figure 2. Fatty acid composition in liver from *Centrophorus* sp. analysed by Peyronel *et al*, 1984, is also included in the figure.

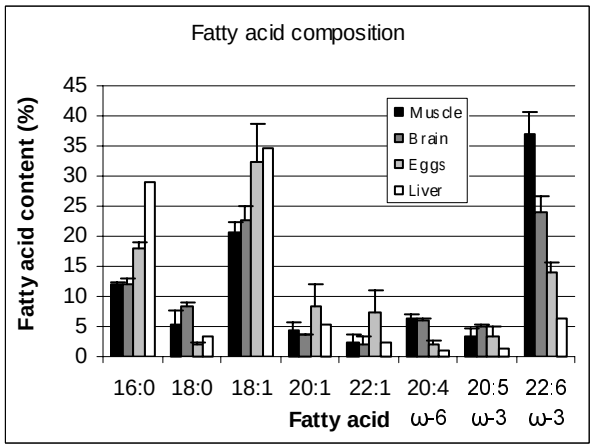

Figure 2: The fatty acid composition for muscle, brain and eggs from *Centrocymnus coelolepis*, *Centrophorus squamosus* and *Centrocyllium fabricii*, and liver from *Centrophorus* sp. analysed by Peyronel et al.

Fatty acid composition of lipid extracts from deep-sea sharks shows a high content of unsaturated fatty acids, especially the monounsaturated fatty acid 18:1 and the polyunsaturated fatty acid 22:6 ω-3. Hazel (1990) have previously found that high content of 18:1 may be an adaptive response to high pressure, and results from Dunstan and others (1988) show low levels of 20:5 ω-3 in cartilagous species compared to bony fish.

The content of squalene in liver has been measured in 10 individuals from each species, and is listed in Table 1. The content varied from 11 % to 84 % among the individuals, and the mean content was 17 % for *C. fabricii*, 48 % for *C. coelolepis* and 66 % for *C. squamosus*.

Table 1: The mean content of squalene in *C. coelolepis*, *C. squamosus*, *C. fabricii* liver oil, including minimum and maximum values.

	Mean ± std (%)	Min (%)	Max (%)
C. coelolepis	48 ± 14	28	67
C. fabricii	17 ± 4	11	23
C. squamosus	66 ± 12	39	84

Antimicrobial activity against *E. coli*, *S. aureus* and *C. albicans*, has been analysed in extracts of subcellular membrane and fatty fractions from liver from *C. coelolepis*, *C. squamosus* and *C. fabricii*. Preliminary results indicate that the activity seems to be correlated with squalamine content in the liver (Shinnar *et al*., 2003). Extract from *C. coelolepis* showed moderate activity (0,05-0,10 µg squalamine) against *S. aureus* and *C. albicans*, while *C. squamosus* had moderate activity against *E. coli*. Lipid extract from *C. fabricii* had no activity against *E. coli*, and weak activity (<0,05 µg squalamine) against the others microbes.

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AOCS, Fatty acid composition by GLC. ALCS Official Method Ce 1b-89

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L 65. VARIATION IN LIPID COMPOSITION IN DIFFERENT REST BIOMASS FROM SPECIES OF THE GADIDAE FAMILY

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Abstract

Marine raw material is characterised by its content of certain n–3 PUFA (polyunsaturated fatty acids) which has well documented beneficial health effects. Today, by-products that are utilised for human consumption is mainly liver and roe, from relatively large cod (*Gadus morhua*). Other cod species, sizes and fractions of rest raw material may also be potential sources of valuable marine lipids [Rustad and Falch, 2002]. The lipid composition and α-tocopherol levels in the liver, total viscera and cut-off of 5 species of the Gadidae family (cod, saithe, haddock, tusk and ling) caught at three different fishing grounds in Europe were evaluated at three different intervals over a one-year period. The weight data from each fraction of by-product was also recorded. The relative weights of the selected parts (head, viscera, liver, cut-offs) and the lipid composition varied between the fishing ground and species. The composition of fatty acids in liver and viscera of haddock were most different from the other species evaluated. Overall, the level of α-tocopherol was reported to be higher in liver than in viscera or cut-off samples and varied more with fishing ground than by season. The variations in α-tocopherol content were not significantly different between neither the fishing ground, species nor season investigated.

Introduction

Globally more than 91 million tonnes of fish and shellfish are caught each year (FAO). Some of the by-products are utilised, but the main part, is more often dumped to waste. Annual discards from the world fisheries are estimated to be approximately 20 million tonnes (25%) [FAO]. In Norway, about 46% of this rest raw material from the cod fisheries (viscera, liver and filleting waste) are utilised [www.rubin.no, 2001], and approximately 34% of these are produced for human consumption. It is therefore a great potential for the fishing industry to utilise more of what is landed. One of the interesting fractions, found in marine biomass, is the marine lipids. Marine lipids have well documented beneficial health effects [Dyerberg and others, 1978; Barlow and Stansby, 1980]. These effects are mainly associated to the long chain highly unsaturated fatty acids EPA (C20:5n-3, eicosapentaenoic acid) and DHA (C22:6n-3, docosahexaenoic acid) which are omega-3 fatty acids specific for marine lipids. Other valuable lipid components found in marine material includes phospholipids, lipid soluble vitamins, sterols and colour components.

In order to utilise the marine lipids found in marine rest raw material, it is a necessity to have control of the variations in lipid content and lipid composition. The chemical composition of fish varies according to factors like age, size, stage of sexual maturity and diet [Dambergs, 1963]. Most of the analytical surveys done on lipid composition in cod, has until now been concentrated on variation within the fillet, which is the part that gives most profit. It has in some work been observed unevenly distribution of lipids and water in different parts of the fillet [Love, 1960, Dambergs, 1964], and the differences has, in some work, been found to be affected by the size of the fish and the spawning cycle. [Love, 1960; Dambergs, 1964; Ingolfsdottir *et. al.*, 1998].

Lean fish species store their lipids mainly in the liver, and mainly as triacylglyserols. In cod liver it has been reported a lipid content of >50% [Shahidi *et. al*., 1991]. The muscle lipids consist mainly of phospholipids, which contains relative higher amounts of polyunsaturated fatty acids than the neutral lipids [Standsby, *et. al*., 1990]. Fatty acid profile (% of fatty acids) has been found to be similar in cod offal and cod liver oils [Shahidi *et. al*., 1991]. While the lipid content in the cod muscle generally is below 1% [Dambergs, 1963], the cut-offs are found to contain higher amounts of lipids compared to the fillet [Shahidi *et. al*., 1991]. The fatty acids in fish oil has been found to vary on the basis of environmental factors like geographic location of catch and season of the year, which, among other factors, has been suggested to be related to the water temperatures [Lovern 1942]. Seasonal variation in the fatty acids of cod liver oil [DeWitt, 1963; Jangaard *et. al.*, 1967] and gonads of cod [Jangaard *et. al.*, 1967] has been presented in the past. The chemical composition of the viscera is varying based on the diet (the composition of the gastrointestinal tract) and spawning cycle. The size of the gonads (milt and roe) of cod vary during the spawning cycle [Eliassen and Vahl, 1982] and will therefore affect the total composition of the viscera. New research on lipid composition in rest biomass in different cod species is lacking.

Material and methods

In this survey, 4 partners have been involved in sample collection, dissection and chemical analysis of viscera, liver and cut-offs from cod species. Cod (*Gadus morhua)*, haddock (*Melanogrammus aeglefinus*), saithe (*Pollachius virens*), tusk (*Brosme brosme*) and ling (*Molva molva*) were collected from the Barents Sea, Icelandic Sea and South Coast of Ireland. The sample collection started in March/April year 2001 and was repeated in June and November the same year. In order to minimize the influence of size, each species got size limitaions for being included in the trial. For each group of fish, a collection of viscera, liver and cut-offs (v-cut, belly flap and muscle from the backbone) from 15 individuals were homogenized and used as a basis for further analysis. All laboratories involved, participated in an intercalibration test before starting on the material from this trial. The test evaluated the analysis of total lipid [Bligh and Dyer, 1959; Folch, et. al. 1957] and the procedure of methylation of fatty acids and the gas chromatograhic analysis of fatty acids. One of the laboratories had different results than the others, but it was decided to use the laboratory closest to the fishing ground and one method for lipid extraction [Bligh and Dyer, 1959] before further analysis of fatty acids, α-tocopherol and lipid classes. The analytical differences, that may have been caused by using different laboratories, have to be taken into account when doing statistical treatment of the results. Weight of the fractions, lipid content, amount of individual fatty acids and α-tocopherol were evaluated and compared.

Data is lacking for some cod species (tusk and ling from the sea around Norway and some cod and haddock from South Coast of Ireland) because of seasonal difficulties in catching. Also fatty acid content from cut-off samples from Iceland is lacking.

Results

The lipid composition varied between the parts that were taken out of the fish. The lipid content in the liver and viscera varied respectively between 42 – 69% and 1.6- 4.6%w/w. The lipid content in the cut-offs samples were higher than usually found in the cod fillet. Since all the analysis are not finished, the statistical analysis was performed on the cod caught in Icelandic Sea and Barents Sea. The analysis of effect showed that some fatty acids were significantly different between species, seasons and fishing ground. These fatty acids were different depending on what variable studied. The test also showed that α-tocopherol was not significantly different between the two fishing grounds, seasons or species analysed, but were significantly different between liver, viscera and cut-off samples.

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L 66. CHARACTERIZATION OF LIPIDS FROM PINK SALMON (*ONCORHYNCHUS GORBUSCHA*) HEADS AND VISCERA, THEIR HYDROLYSATES AND DRIED MEALS

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Abstract

Alaska still has abundant wild commercial salmon landings with nearly 300,000 metric tons (mt) of Pacific salmon (*Oncorhynchus* spp.) landed last year (2002). We estimate roughly 50,000 mt of salmon heads and 30,000 mt of viscera are among the byproducts of Alaskan salmon processing each year. The objective of this study was to characterize the lipid fraction from both pink salmon heads and viscera in raw samples in wet and dried hydrolysates. Commercial processors in Kodiak provided 1,000 kg of fresh pink salmon (*Onchorhynchus gorbuscha*) heads and viscera. These were immediately processed in a pilot plant sized hydrolysis unit at the Fishery Industrial Technology Center. Heads or viscera were ground, mixed with commercial papain and hydrolyzed at ~65°C. At the completion of hydrolysis, the enzyme was denatured at ~85°C and the hydrolysates concentrated in an evaporator to ~45% solids. Subsequently, hydrolysates were dried using a Littleford dryer and mixed with ethoxyquin to 150 ppm for storage. Lipid content, fatty acid profiles and lipid classes were determined on samples of raw material, wet and dried hydrolysates. Fat content in whole pink salmon varies seasonally, ranging from 2 to 8%. Extracted lipids were analyzed on an Iatroscan MK-6s to measure the concentration of triglycerides, diglycerides, monoglycerides, free fatty acids, sterols and phospholipids as milligrams per gram of oil. The results show free fatty acid content higher in pink salmon viscera and its hydrolyzed products, while pink salmon heads and its hydrolyzed products had higher triglyceride content. Fatty acid methyl esters were prepared and fatty acid profiles determined on an Agilent GC6850. These results demonstrate significant differences between pink salmon heads and viscera in saturated / polyunsaturated and the omega 3 omega 6 fatty acid ratios.

Mateials and methods

Lipid Extraction, Fatty Acid Methyl Ester (FAME) Analysis and Gas Chromatography

Lipid extraction was carried out in triplicate, preparation of fatty acid methyl esters was carried out by the method of Maxwell and Marmer (1983), and gas chromatographic identification of fatty acids was performed as described by Smiley et al (2003).

Lipid Class Analyses. Six standards, purchased from Sigma (St. Louis, MO.), were used to identify the lipid classes found in pink salmon oil: cholesterol (ST), triolein (TG), oleic acid (FFA), phosphatidylcholine (PL), diolein (DG), and monopalmitoylglycerol (MG). A five-point calibration curve was determined for each. Hydrogen flow was 60ml/min and airflow 1.6 L/min. The solvent system was hexane: ethyl ether: formic acid in the ratio of 80:25:1.2 (Ackman et al.,1990). Chromarods-SIII (Iatron Labs, Inc., Tokyo, Japan) were conditioned for spotting. 10 µl of 1 mg lipid per 1 ml chloroform was spotted on each rod. Spotted rods were suspended in the solvent chamber for 10 minutes, eluted for 30 minutes, oven dried for 3 minutes at 110°C, and then scanned (Whitsett et al., 1986). After use, rods were cleaned overnight in concentrated chromic H_2SO_4, rinsed with distilled water, soaked for 3 hours in 5% ammonium hydroxide, rinsed several times with distilled water followed by a final acetone rinse and oven dried for an hour. Clean rods were stored in a 30% humidity chamber until analysis (Parish, 1987).

Statistical Analysis. The weighted means are derived from an analysis of variance run on Statistica version 6.0 (StatSoft, Inc.). For tests of statistical significance, the data was subjected to analysis of variance followed by a Bonferroni post-hoc test with significance set at $p<0.05$. Those means labeled a, b, c and d are statistically distinct from each other at the $p<0.05$ level.

Results and discussion

We analyzed replicate samples from the raw material, crude hydrolysate and dried meal derived both from pink salmon viscera and heads. We do not report our analysis of oil from raw viscera or heads for individual fatty acids because of degradation problems encountered during storage. Table 1 shows free fatty acid content is significantly higher in oil from raw pink salmon viscera and its wet hydrolysate. Oil from pink salmon heads had significantly higher triglyceride content than was found in viscera. Table 2 summarizes pertinent information from the fatty acid methyl ester analyses. Omega 3 and 6 fatty acids are significantly more abundant in salmon heads than in the viscera, and yet there is no significant difference in the ratio of ω3 to ω6 fatty acids between these

tissues. Levels of saturated, monounsaturated and polyunsaturated fatty acids are significantly higher in heads than viscera. Yet, the ratio of polyunsaturated to saturated fatty acids remains similar in both tissues. We identified differences in the amount of mean saponifiable fatty acids between heads and viscera. Yet, in both tissues, C 16:0, C18:1ω6 cis, C20:1ω11, C20:5ω3, C22:1ω11 and C22:6ω3 fatty acids are the most abundant and found roughly in the same order of abundance. The concentrations of most of the individual fatty acids, as determined from their methyl esters are similar in these tissues but numerically lower in viscera. The differences reported here may well be due to endogenous enzyme activity in the viscera.

Table1. Lipid Class Analyses (mg/g oil)

PINK SALMON	VISCERA			HEADS		
LIPID CLASS	Raw Material	Hydrolysate	Fish Meal	Raw Material	Hydrolysate	Fish Meal
% Lipids	8.88 a	3.28 b	12.78 c	8.81 a	7.22 a	29.82 d
Mean TG's	172.82 a	213.13 a	181.68 a	411.79 b	365.44 c	466.94 c
Mean FFA's	365.85 a	207.37 b	161.6 c	154.19 c	142.73 c	115.01 d
Mean DG's	76.40 ac	80.03 ab	69.47 ac	91.45 b	77.02 a	64.28 c
Mean ST's	187.07 a	207.24 a	157.38 b	109.07 c	141.22 bd	119.25 cd
Mean MG's	164.02 a	145.73 b	126.67 c	130.50 cd	138.96 db	114.42 e
Mean PL's	167.84 a	146.47 b	177.16 a	136.56 bc	134.60 c	120.08 d

Lipid Classes: TG triglycerides, FFA free fatty acids, DG diglycerides, ST sterols, MG monoglycerides, PL phospholipids

Table 2. Summary Fatty Acid Methyl Ester GC Analysis (mg/g oil)

PINK SALMON	VISCERA		HEADS	
FATTY ACID SUMMARY	Hydrolysate	Fish Meal	Hydrolysate	Fish Meal
Mean Ω 3 FA's	59.68 a	99.35 b	112.59 b	145.58 c
Mean Ω 6 FA's	4.20 a	3.79 a	9.46 b	12.82 c
Mean Ratio Ω3:Ω6	14.42 a	26.23 b	11.99 a	11.37 a
Mean Monounsaturated	147.95 a	105.53 b	272.66 c	253.74 c
Mean Saturated (S)	89.47 a	87.89 a	146.24 b	136.62 b
Mean Polyunsaturated (P)	48.22 a	114.35 b	120.65 b	169.06 c
Mean Ratio P:S	0.54 a	1.30 b	0.83 c	1.24 b
Mean Saponifiable FA's	303.04 a	326.12 a	597.38 b	634.63 b
Mean Identified FA's	296.04 a	325.58 a	587.09 b	614.50 b
∑ Reported FA's	285.65 a	307.77 a	539.55 b	559.42 b
∑ FA's < 2 %*	17.39 a	18.35 a	58.83 b	75.21 c

* Identified but not reported

Table 3. Fatty Acid Methyl Esters GC Analysis (mg/g oil)

PINK SALMON	VISCERA		HEADS	
FATTY ACID PROFILE	Hydrolysate	Fish Meal	Hydrolysate	Fish Meal
Mean C14:0	16.26	10.14	31.17	28.85
Mean C16:0	55.81	53.86	95.69	89.33
Mean C16:1ω7	19.19	12.49	27.3	25.41
Mean C18:0	17.39	23.89	19.38	18.43
Mean C18:1ω9 cis	67.18	52.98	83.93	78.63
Mean C18:1ω7	15.31	15.36	17.39	16.75
Mean C18:2ω6 cis	4.20	3.79	9.46	12.82
Mean C18:4 ω3	2.26	2.76	7.42	14.60
Mean C20:1ω11	17.87	9.32	55.05	50.68
Mean C20:1ω9	9.16	7.06	20.80	19.30
Mean C20:5ω3	16.30	37.35	41.60	55.14
Mean C22:1ω11	19.23	8.32	68.20	62.97
Mean C22:5ω3	6.25	14.07	8.88	14.29
Mean C22:6ω3	19.21	56.39	53.28	72.21

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K 7. ENZYMES AND BIOACTIVE PEPTIDES FROM FISH WASTE RELATED TO FISH SILAGE, FISH FEED AND FISH SAUCE PRODUCTION

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Abstract

This paper gives a brief survey of research and developments concerning enzymes and bioactive peptides from fish waste or by-catch applied for fish silage, fish feed or fish sauce production. A new fish silage processing technology invented in Norway in the late 1970-ies revealed the possibility of recovering pepsins and bioactive peptides from fish silage. Whereas the pepsins are used for gentle bioprocessing of certain fishery products, the peptides may be valuable supplements in formulation of fish feed. Both *in vitro* and *in vivo* studies have shown that certain peptide fractions in fish protein hydrolysates stimulate the non-specific immune defence system in fish. Fish sauce and fish silage are protein hydrolysates occuring as a result of autolytic digestion, but whereas fish silage is obtained by rapid peptic digestion at acid condition, fish sauce develops by slow tryptic digestion at neutral and very salt conditions. Generally fish sauce is regarded as a typical Asian product made from tropical fish species, but resent studies have shown that fish sauce can be made also from cold water species.
Keywords: fish silage, fish sauce, enzymes, bioactive peptides

Introduction

During the initial period of industrialized fisheries fish resources appeared to be inexhaustible, and byproducts were looked upon as worthless garbage. However, it was soon acknowledged that this garbage became a problem when it started to accumulate in every fishing harbour. In Norway this was first recognized as a serious problem in the early 1970-ies, and this recognition initiated a gowernmental applied research program for value addition of fishery by-products. A vital element in this work was the invention of new technology for fish viscera silage processing where a highly nutritious hydrolysate concentrate very suitable as a protein supplement in animal feed could be recovered (Raa and Gildberg, 1982). By minor modifications the technology could also be used to recover crude fish pepsins and the low molecular weigth peptone fraction by ultrafiltration (Gildberg and Raa 1977, Gildberg and Almås 1986, Gildberg 1992). Whereas pepsins from cold water fish are active at low temperature (Gildberg 1988) and suitable for gentle enzymatic processing of some fishery products (Gildberg 1993) the hydrolysate fraction has a high nutritional value and also contains immuno stimulating peptides (Almås 1990, Gildberg and others 1996). Figure 1 briefly illustrates the procedure for recovery of oil, pepsins and a low molecular weight protein hydrolysate (peptone) from a silage of cod viscera and indicates some possible biotechnological applications of the products.

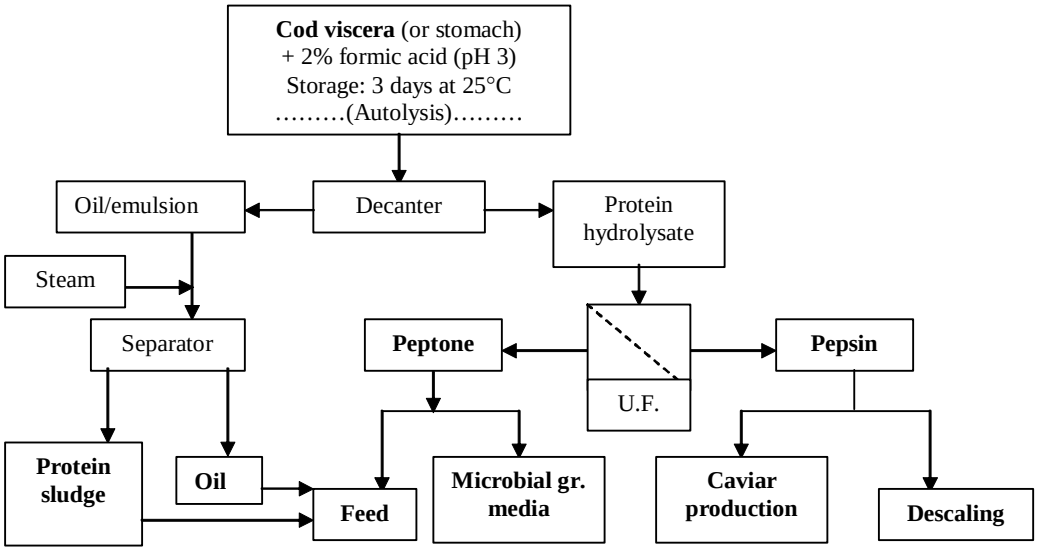

Figure 1. Flowchart illustrating the possibility of processing cod viscera silage to generate useful products for biotechnological applications.

During the 1980-ies this simple procedure for recovery of pepsin and peptone became a small scale industry in Norway, and a number of applications were tested out. A few of them still remains viable today 20 years later.

Recovering pepsin and peptone from fish silage

A fish silage made from a raw material with a high pepsin content will quickly solubilize. Carnivorous fishes, like Atlantic cod, has a high stomach pepsin content all year around, and a silage made from minced viscera, or even better, the separated stomach, will solubilize completely after a few days of storage. Optimal storage conditions for the recovery of pepsins are pH 3 and about 25°C for 3 days (Gildberg 1992). By ultrafiltration, concentration and spraydrying a cod stomach silage may provide a crude pepsin preparation corresponding to 0.5-1 g pure pepsin per kg. The purity of the crude preparation may vary in the range 2-10% depending on the raw material and washing procedures during ultrafiltration. A cod viscera silage will give only ¼ of the pepsin amount, but silages of both stomach and viscera will provide about 100 g low molecular weight peptone per kg raw material. The peptone fraction may easily be concentratet to 40-50% dry matter by vacuum evaporation. This acid concentrate is quite stable and can be stored for several months without deteriorating, but neutralisation and spraydrying is recommended since most customers prefer dry products.

Application of fish pepsins

Since Atlantic salmon pepsin was first purified by crystallisation in the late 1930-ies (Norris and Elam 1940) various fish pepsins has been thoroughly studied (Gildberg 1988), and a preliminary culimination of the research efforts was achieved by the determination of the complete amino acid sequence and the crystalline structure of an Atlantic cod pepsin in 1998 (Karlsen and others 1998).

Generally fish and mammalian pepsins are structurally quite similar, but there are some important differences which are related to pH and temperature characteristics. Particularly pepsins from cold water fish species ar more active at low temperature and less stable at elevated temperatures than mammalian pepsins. They also have optimal activity at less acid conditions. These differences make fish pepsins more useful than mammalian pepsins in situations were processing at gentle physical and chemical conditions is required.

In the 1980-ies the application of fish pepsins as rennet substitutes in cheese production was thoroughly investigated (Brewer and others 1984, Haard 1986, Guerard and LeGal 1989). Although it was possible to produce good quality cheese using various fish pepsins, such production was never commercialized. Possibly because the industry developed recombinant chymosin production during the same period.

In Norway the use of cod pepsin for gentle processing of some fish raw materials were tested out. Joakimson (1984) showed that cod pepsin could be used for deskinning of herring, but due to variable skin structure and thickness at different parts of the fish, it was difficult to obtain uniform deskinning without damaging the muscle. Better results were obtained when cod pepsin was used for descaling. After gentle treatment of fishes like hake and haddock with pepsin at weak acid conditions, the scales could easily be removed by a rapid passage through a water jet system (Svenning and others 1993). Industial equipment for enzymatic descaling has been developed and the technology is commercialized.

Traditionally mechanical rubbing is used to release the roe from connective tissues during production of caviar from troat and salmon. Normally about 30% of the roe is damaged by such processing. The Norwegian company Biotec AS has developed a gentle technology were the roe is released by floatation in a tank after pepsin treatment. By employing this technology, a caviar recovery of about 90% may be achieved (Raa 1990).

Application of peptones made from fish viscera silage

The soluble protein fraction of fish viscera silage is rich in essential amino acids and has a high nutritional value (Strøm and Eggum 1981). Due to a low average molecular weight, it is suitable as an easy digestible protein supplement in the feed to juvenile fish and domestic animals (Raa and Gildberg 1976, Raghunath and Gopakumar 2002). An exciting experience from supplementing fish viscera silage proteins to ruminats in Norway has been that it seems to improve the animal health situation. These observations has not been scientificly doccumented, but both *in vitro* and *in vivo* experiments with fish indicates that a low molecular weight peptide fraction from a silage of cod stomach stimulates non-specific immune responce reactions and improves the disease resistance in fish (Gildberg and others 1996, Gildberg and Mikkelsen 1998). Similar results have been achieved with low molecular weight proteins from cod milt (Pedersen and others 2003).

Fish viscera peptone is also an excellent nitrogen source in microbial growth media. It has been used succesfully in cultivating fish pathogens like *Vibrio salmonicida* for vaccine production, and it has been shown that it provides better growth than high quality peptones like Bacto Tryptone and Bactopeptone with several microorganisms of commercial interest (Almås 1990, Clausen and others 1985, Vecht-Lifshitz and others 1990).

Fish sauce

Although fish sauce apparently originated in Southern Europe more than 2000 years ago (Corcoran 1963), it wanished from the Western World several hundred years ago. At present a revival of the product is occuring both in Europe and America. In Southeast Asia, however, fish sauce has been acknowledged as a popular food item for centuries (Saisithi 1994).

Fish sauce is made from small pelagig fish species, by-products or by-catch fish by salt fermentation. After tank storage of a fish:salt mixture (3:1) at tropical temperatures for at least a half year, an amber protein solution is drained out from the bottom of the tank. This delicious liquid is fish sauce. Although fish sauce is used as a condiment on vegetable dishes, it is also a rich source of essential amino acids to a great number of people normally pulling through mainly on vegetable foods. A high value in methionine and particularly lysine in fish tissues provides a good balance to most vegetable proteins.

Although a number of exciting nutraceutical properties, including profylactic effects against gastrointestinal diseases and curative effects after both external and internal medication have been attributed to fish sauce (Saisithi 1994, Corcoran 1963), little has been done to verify medical properties of fish sauce. Recently, however, stimulation of the proliferation of human white blood cells was obtained during *in vitro* cultivation with peptides from fish sauce (Thongthai and Gildberg, 2003). A significant growth stimulation was obtained in the precence of 5 µg/ml of small size fish sauce peptides, whereas no stimulation was detected at higher or lower concentrations. Whether this observation reveal an important health aspect connected to the consumption of fish sauce remains to be verified. However, the general tendency of fish sauce being a new exciting food item in the Western World is undoubtible. Table 1 reviewes resent research on fish sauce in Europe and Northern America.

Table 1. Resent research on fish sauce in Europe and North America

Author(s)	Year	Species	Topic
Raksakulthai N, Haard NF	1992	capelin	Correlation between the concentration of amino acids and the flavour of fish sauce
Pedersen G, Skjerdal T	2000	cod, saith	Utilisation of waste from the salt fish idu-stry in production of fermented fish sauce
Gildberg A, Thongthai C	2001	sprat	Effect of reduced salt and addition of lactic acid bacteria on quality of fish sauce
Gildberg A	2001	capelin	Supplementing capelin with cod intestines
Aquerreta Y, Astiasaran I, Bello J	2002	tuna, mackerel	Use of exogenous enzymes to elaborate the Roman fish sauce “garum”
Lopetcharat K, Park JW	2002	whiting	Fish sauce made from pacific whiting and surimi by-products during fermentation

Several research groups, as well as industry applicators, are showing great interest in this topic, and due to the limited amount of suitable raw material in the east, the interest for establishing production in the west is increasing. Promising results have been obtained with fish sauce produced both from cold and temperate water species, but still a large scale industrial production of fish sauce has not been established in the Western World.

Conclusion

The work on fish viscera silage has revealed that the pepsins can be recovered by ultrafiltration. The pepsins from cold water species like Atlantic cod have proved to be useful in caviar production and in descaling of fishes like hake and haddock. Certain peptide fractions from fish silage stimulates the non-specific immune defence system in fishes and possibly also in warm blooded animals. Apparently fish sauce contains similar peptides and may soon become a new exiting neutraceutical food of the Western World.

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L 67. ANAEROBIC DIGESTION OF FISH WASTE-A NOVEL SOLUTION TO THE PROBLEM?

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Abstract

Background

For decades, wastes from the fisheries industry have been dumped at sea or in landfills. These practices were not ecologically viable nor did they utilise the energy potential of these wastes. With the prohibition of marine dumping and the move towards reuse and recycle, other treatment options have to be explored. Treatment by anaerobic digestion may be a feasible option, incorporating waste utilisation and energy production in the form of biogas. The advances in anaerobic digestion over the last 40 years have made the process an extremely attractive option for the treatment of highly polluted wastewaters.

Objective

The study involves the operation of three laboratory scale anaerobic reactors (upflow anaerobic sludge blanket) each operating at different temperatures. The primary objective of the study is to establish the optimum conditions necessary (both temperature and reactor configuration) for treatment of the fish waste to the required level. Examination of the problems associated with the anaerobic digestion process (i.e. toxicity of sodium, oleic acid etc) that would prevent its implementation in the fisheries industry will be a secondary aim of the study.

Methodology

Standards methods for the examination of wastewater will be used for the duration of the study. Figure 1 below illustrates the reactor design that will be used throughout the duration of the study. As the study is in its infancy, results are not available at present.

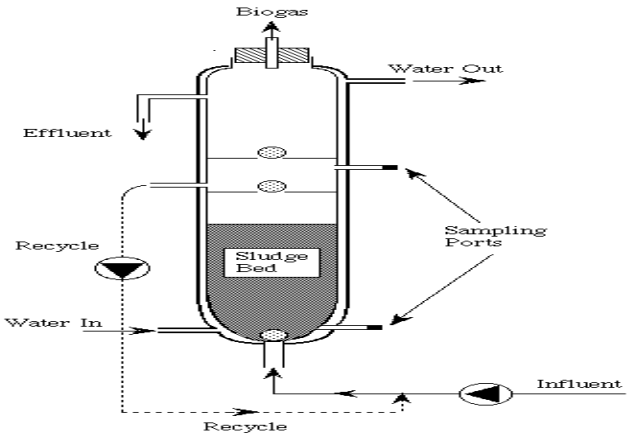

Figure 1. Upflow anaerobic sludge blanket (UASB)

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L 68. HOW ENZYMES MAY BE HELPFUL FOR UPGRADING FISH BY-PRODUCTS: ENHANCEMENT OF FAT EXTRACTION

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Abstract

These results are part of the EU-project "Utilisation and stabilization of by-products from cod species". The main objective of this work is to increase the utilisation of by-products from cod species to produce value added food ingredients. This project will focus on the following species of Gadidae/cod: cod (*Gadus morhua*), saithe (*Gadus virens*), haddock (*Melanogrammus aeglefinus*), tusk (*Brosme brosme*), ling (*Molva molva*). One of the role of the Laboratory of Food Engineering of IFREMER is to provide information on the lipidic composition of the by-products from those species and also to study how to improve their added value. One of the way retained is to focus on the lipidic extraction from such biomass. Indeed, data from characterization of lipid in some by-products (liver, roe, viscera, head, milt, backbone and cut-offs) from those cod species has shown that these parts can be utilized as a raw material for production of lipidic compounds of interest such as fish oils for human consumption. Nowadays in industry, fat extraction is carried on at high temperature which can lead to some lipid oxidation and very few work is done for optimizing those extractive conditions. Our experiments have focused on the use of enzyme for maximizing the fat extraction from cod by-products. Experimental design were used for finding optimal conditions.

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K 8. MARINE BIOTECHNOLOGY

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Introduction

Biotechnology is a term with a myriad of definitions. It can be defined as the application of scientific and engineering principles, such as, cell biology, biochemistry, microbiology, molecular biology, and molecular genetics to the processing of materials by biological agents to provide goods and services (Ahmed and Attaway, 1987; Osinga *et al*., 1999a; IFT, 2000); or as the means of manipulating life forms (organisms) to provide desirable products for man's use (Nill, 2001). The U.S. Department of Agriculture (1990) has defined it as "the use of living organisms, cells, subcellular organelles and/or parts of those structures, as well as the molecules, to effect biological, chemical or physical changes". Although biotechnology has been used by man for thousands of years in the production of food, it has come into the forefront within the last three decades through the manipulation of genes and transfer of genetic material to develop new products or system pathways. Applications of marine biotechnology are found in medical/pharmaceutical applications, as well as the production of food and chemicals (Osinga *et al*., 1999a). This paper will give an overview of marine biotechnology and review the latest research in the controlled manipulation of marine organisms (vertebrates or invertebrates) to produce functional and valuable components and discuss the use of waste materials as a resource for bioactive compounds.

The unique characterisitics of the marine environment sets it apart in the growing field of biotechnology. The marine area covers close to three-quarters of the earth's surface representing over 90% of all water in the hydrosphere. It is one of the largest underutilized biological resources providing an immense array of organisms with unique structures (Gutrich *et al*., 1998) and characteristics (i.e., halotolerant, barotolerant, thermophiles and psychrophiles), metabolic pathways, reproductive and sensory systems, as well as defense mechanisms. These are currently being studied by geneticists and molecular biologists to find useful enzyme and bioactive components for the production of fine chemicals for the food and pharmaceutical industries (Powers, 1990). Although the application of biotechnology in the marine environment has been limited compared to the terrestrial systems, there have been some notable successes indicating that the entire field of marine biotechnology is now a new major frontier of scientific discovery. The strategy to be followed in diverse fields, such as, aquaculture, *in vitro* culture, gene transfection, or enzyme engineering, depends on both the availability of the organism and the biosynthetic pathway of the target metabolite (Osinga *et al*., 1999b). This paper investigates new studies as well as trends of marine biotechnology research focusing on the innovative molecular genetics techniques related with aquaculture, bio-processing, natural products and biomaterials that can aid food and pharmaceutical industries as well as other fields. Novel tools and processes are required for identification of new products, optimization of production, efficient product recovery, and use of the resources for sustainable development.

Biotechnology in Aquaculture

As the world population increases, so does the demand for high-quality protein, such as, protein from aquatic food products. While global capture fisheries have leveled off in the last decade, aquaculture has increased steadily and raised the potential to meet the world's needs for high quality protein (Powers, 1990; NACA/FAO, 2001). According to the FAO (2000) the world fishing production was 126.2 million tons, of which 25% was derived from aquaculture. It is predicted that by the year 2030 aquaculture will dominate the fish supplies and less than half of the fish consumed will likely originate from capture fisheries. With this paradigm shift in aquatic food production, researchers will have to find new and safer ways of producing food. Many agencies in both developed and developing countries are looking toward biotechnology to provide some of the answers. Biotechnology has been used as a powerful tool in the past two decades, making major contributions in disciplines of biomedical sciences, agriculture and pharmaceutical industries. With the help of biotechnology, aquaculturists can have genetically modified or transgenic organisms (GMOs) with increased growth rate, improved nutrition of feeds and feed conversion. Biotechnology will allow scientists to produce vaccines for farmed species so fish can grow healthier, faster and disease-free, extending the range of aquatic species being farmed. Certainly, this is not without controversy and the use of GMOs in aquaculture as in the production of terrestrial farm animals is moving slowly. One of the major concerns with GMO aquatic animals is escapement. Unlike their terrestrial counterparts, aquacultured species can more readily escape into the wild and have potential interaction with native species. This and the rising concern of GMOs in the food chain has slowed research of GMOs and their potential use for food production. Table 1 (FAO 2000) shows some aquatic GMOs being tested for use in aquaculture. The major technologies currently used to reach these goals in are: a) transgenic technology to generate animals with defined phenotypes, also used as bioreactors for the production of therapeutic proteins for human health, and embryonic stem cell technology for gene targeting trapping and insertional mutagenesis, b) protein expression technology

which refers to the production of recombinant proteins with the proper folding, modification, and activity (bioactive molecules such as hormones, gonadotropins, and enzymes for the use in aquaculture and/or food production; c) genomics to identify and characterized genetic markers important for drug discovery; d) DNA vaccines to combat disease, e) proteomics to produce and identify proteins that are expressed/altered under experimental conditions targeting therapeutics and drug discovery; and f) Restriction Fragment Length Polymorphism and Quantitative Trait Loci used primarily for stock identification breeding selection, and the identification of genetic markers for growth enhancement and disease resistance (Hew and Fletcher, 2001). Research using biotechnology for aquaculture feeds to improve growth, increase disease resistance, and improve environmentally sound practices for the development of sustainable aquaculture programs (Garcia-Carreno and Vargas-Albores, 2002).

Transgenic technology and protein expression

The production of transgenic fish is a potent means to improve the performance of many farmed fish. This technique is generally more effective than traditional breeding and offers a direct and selective method for introducing well-defined DNA encoding of a gene product for a specific biological function into the genome of the recipients (Hew *et al*., 1998). Fish species are generally very tolerant to artificial manipulation of their chromosomes during early

Table 1. Some aquatic GMOs being tested for use in aquaculture[a]

Species	Foreign gene	Desired effect	Country
Atlantic salmon	AFP (antifreeze protein) AFP salmon GH	Cold tolerance Increased growth and feed efficiency	United States, Canada
Coho Salmon	Chinook salmon GH + AFP	After 1 year, 10- to 30 fold growth increase	Canada
Chinook salmon	AFP salmon GH	Increased growth and feed efficiency	New Zealand
Rainbow Trout	AFP salmon GH	Increased growth and feed efficiency	United States, Canada
Cutthroat Trout	Chinook salmon GH + AFP	Increased growth	Canada
Tilapia	AFP salmon GH	Increased growth and feed efficiency: stable inheritance	Canada, United Kingdom
Tilapia	Tilapia GH	Increased growth and feed efficiency: stable inheritance	Cuba
Tilapia	Modified tilapia insulin-producing gene	Producing of human insulin for diabetics	Canada
Salmon	Rainbow trout lysosome gene and flounder pleurocidin gene Mx gene[b]	Disease resistance, still in development	United States, Canada
Striped bass	Insect genes	Disease resistance, still in early stages of research	United States
Mud loach	Mud loach GH + mud loach and mouse promoters genes	Increased growth and feed efficiency; 2- to 30 fold increase in growth; inheritable transgene	China, Korea Rep.
Channel catfish	GH, Silk moth cecropin + promotor[c]	33% growth improvement in culture conditions Bactericidal activity	United States
Common carp	Salmon and human GH, rainbow trout GH[d]	150% growth improvement in culture conditions; improved disease resistance	China, United States
Indian carps	Human GH	Increased growth	India
Goldfish	GH AFP	Increased growth	China
Abalone	Coho salmon GH + various promoters	Increased growth	United States
Oysters	Coho salmon GH + various promoters; Neo^R bacterial gene[e]	Increased growth Resistance to antibiotics, still in development	United States
Medaka[f]	Silk moth cecropin gene + promoters	Bactericidal activity	United States
Sea bream[g]	Rainbow trout GH + promoter	Increased growth	China, United States

AFP = anti-freeze protein gene (Arctic flatfish)
GH = Growth hormone gene
[a] From FAO, 2000.
[b]Jensen *et al*., 2002.
[c]From Dunham *et al*., 2002a.
[d]From Dunham *et al*., 2002b.
[e]From Buchanan *et al*., 2001
[f]From Sarmasik *et al*., 2002.
[g]From Lu *et al*., 2002.

development, a characteristic that has been exploited for the production of inbred lines, monosex populations, and the control of ploidy (Foresti, 2000). This technology can be used to: improve growth rate of fish; control sexual maturation, sterility and sex differentiation; improve survival by increasing disease resistance against pathogens; adapt to extreme environment, such as, freeze/cold resistance; and alter the biochemical characteristics or metabolic pathways for enhancing nutritional qualities of fish flesh and improving food utilization (Hew and Fletcher, 2001). To date, a large number of fish species have been genetically modified using different DNA techniques (Table 1), reflecting the extensive application of this approach on fish aquaculture. Nevertheless, a major concern is the potential negative impact that this technology could have, such as in markets and the danger of possible interaction of this transgenic fish with the wild population which still has to be assessed (Foresti, 2000).

Growth hormone (GH) and bactericidal gene transfection

Growth hormone research dates back to 1985, when Professor Zu Yan Zhu astonished researchers throughout the world by reporting that his group had transferred a human GH gene into fish (Powers, 1990). Since then, much work has been done in transfecting (insertion of DNA genes into cells) economically important species. Fish species are generally very tolerant to artificial manipulation of their chromosomes during early development, and this has been used to exploit the production of inbred lines, monosex populations, and the control of ploidy. This is of interest because, by controlling ploidy, researchers control sterility which has important implications to prevent interactions due to escapement and thus fisheries management. Female triploids have been found to be superior to males for carcass quality (Foresti, 2000).

GH is involved in the regulation of somatic growth and maintenance of protein, lipid carbohydrate and mineral metabolism as well as improving appetite, feeding efficiency and growth rate (Hsih *et al*., 1997). The objective of most aquaculture operations is to grow organisms to a size and quality suitable for human consumption. Because animal diseases account for a major loss of revenue and often make the difference between success or failure of a commercial endeavor, marine biotechnology has focused on insertion of GH and bactericidal genes to best meet production goals. Buchanan et al. (2001) documented the first successful insertion and expression of a foreign DNA to increase antibiotic resistance in eastern oyster larvae and, in this way, prevents diseases that affect the species. Although the technique has been proven successful, it still needs to be improved for selection of transgenic larvae and its survival. Guillen *et al*. (1999) evaluated the safety of eating transgenic tilapia with accelerated growth in human healthy volunteers and found that transgenic tilapia flesh was well accepted and no differences were found in the clinical and biochemical parameters evaluated. This pioneer study suggests that transgenic tilapia (and probably most transgenic species) can be safely consumed. However, as Braun states (2002), all technologies have occasional failures that can lead to accidents, in other words, there is no "zero risk" for human activities. A challenge for marine biotechnologists (and a totally different approach to achieve fish growth) is through the use of probiotics and microbial feed additives that have shown great benefits for terrestrial livestock. In preventing the colonization of pathogens, probiotics may act to improve the fish growth efficiency as pathogens impair digestive functions and direct the organisms energy into fighting disease (Lyndon, 1999).

Antifreeze protein (AFP) or ice structuring proteins (ISP)

Fish are found in all waters, from the tropics to Antarctica, and temperate waters where thermal conditions vary throughout the day and among seasons. Most fish, due to the presence of sodium chloride and other small molecular weight electrolytes, have a freezing temperature of approximately –0.6°C. Nevertheless, temperatures in polar regions can reach as low as –1.9°C, requiring special mechanisms for survival (Hew and Fletcher, 2001). Fish living in the polar regions have developed a unique group of proteins named antifreeze proteins (AFP) or antifreeze glycoproteins (AFGP), or more recently ice structuring proteins (ISP) (Tharp and Young, 2003). These proteins have the ability to interact with ice crystals, binding to surface areas, modifying their structure and effectively inhibiting crystal growth. This lowers the freezing point of blood preventing freezing and protecting membranes from cold damage allowing the fish to survive subzero temperatures (Ben *et al*., 1999; Eniade *et al*., 2001; Evans and Fletcher, 2001). There are 4 types of AFP (types I-IV based on their structural features) and one type of AFGP ranging in concentration from 10-25 mg/mL (Hew *et al*., 1998; Low *et al*., 2001). Studies have shown the successful incorporation of the AFP gene into salmon genome. However, due to the lack of the processing enzymes necessary for the maturation of AFP, it only generates pro-AFP with a 70% activity compared to the mature polypeptides. Improvements to enhance the antifreeze level of transgenics, thus insuring aquaculture in northern waters, are underway (Hew and Fletcher, 2001). The AFP gene has also been employed as vector or promoter for its utilization in gene transfer studies such as in the production of an AFP-GH chimeric gene. Since most commercial fish do not possess this gene, its detection by PCR is simple, making it a valuable marker in these types of studies (Hew *et al*., 1998; Hew and Fletcher, 2001).

Besides the aquaculture application of antifreeze proteins, these proteins have generated considerable interest among researchers in the medical area, for application such as cryopreservation of transplant organs and cells, and cryosurgery (Crevel *et al*., 2002); as well as with food technologists for improving the properties of frozen meat (Payne and Young, 1995) and ice cream (Li and Lee, 1995; Feeney and Yeh, 2000). These studies have shown a promising use of AFP in foods, however, its utilization still remains pending due to the high costs of production in addition to consumer's perception. Possible ways to reduce costs could be through production of analogs or chemical synthesis of these types of proteins (Ben *et al*., 1999; Feeney and Yeh, 2000; Eniade *et al*., 2001) or genetically introducing these proteins into foods through genetic engineering (Feeney and Yeh, 1993; Li and Sun, 2002). The public's concerns and perception about allergenicity to consumption of these types of proteins has been addressed in several papers, showing that they are unlikely to present a potential sensitization hazard

(Baderschneider *et al*., 2002; Crevel *et al*., 2002; Bindslev-Jensen *et al*., 2003) therefore they should be considered as a future additive for foods.

Marine Biotechnology and Production of Bioactive Substances

The world's oceans represent one of the largest unexploited biological resources. Nevertheless, the marine environment has proven to be a rich source of both biological and chemical diversity. It is estimated that only a small percentage of the total number of species has been discovered (Pomponi, 1999). New bioactive substances discovered from marine species and the diversity of marine life suggests that an immense number of new bioproducts can be discovered and developed commercially. Numerous potential pharmaceuticals (e.g. mololipids, an anti-HIV compound), useful energy producing bacteria (e.g. photobiological hydrogen production), functional biopolymers (e.g. chitin), a number of products for the food industry (e.g. new vitamins, α-tocomonoenol; carotenoids, polyunsaturated fatty acids or PUFAs), biomimetic approaches in materials science (e.g. synthesis of silica structures from sponges) have been found in and isolated from marine organisms. Recently, approaches have been taken in bridging the divide between marine technology and bioprocess engineering to stimulate research that can lead to commercial and sustainable exploitation of these natural resources (Osinga *et al*., 1999a). New bioengineering methodologies are being developed for the expression of marine bacterial natural products in a terrestrial heterologous host, demonstrating that marine bacterial natural products can be produced in non-marine expression systems, showing a great potential for mass production (Moore and Piel, 2000; Piel et al., 2000).

Marine algae

Of all plant species of the world, some 30,000 species belong to the single oxygen evolving photosynthetic thalloid forms called algae or seaweeds. These organisms, besides supplying oxygen to the biosphere, are also sources of food to fish and man (Garg, 1994). These are divided in micro and macroalgae. Microalgae are the most primitive and most simply organized members of the plant kingdom. The majority exists as small cells of about 3-20 μm, with a few species organized into simple colonies. Macroalgae or seaweeds have developed a level of organization with cells grouped into structures that resemble the leaves, stems, and roots of higher plants (Arad and Spharim, 1998). Bioactive substances derived from algae species have been researched for several years with different degrees of success. Some well-established industries are exploiting algal resources for food additives production as well as for fluorescent dyes for use in the pharmaceutical industry and as diagnostic markers. The production of carrageenan, agars and other marine polysaccharides is well documented and examples of successful commercial operations. Nevertheless, there is potential for numerous other bioactive products to be discovered and commercially exploited.

The amount of bioactive substances (carotenoids, PUFA's, etc) accumulated for the algae is a function of several operating variables that may be used to enhance the production of such compounds to have success in a commercial scale. The main constraint for the commercialization of new microalgae and microalgal products is the need for closed culture systems called photobioreactors. These systems have high costs of operations due to high light requirements and slow growth rate of these microorganisms (Borowitzka, 1999; Molina-Grima *et al*., 1999). There is still a need for the development of versatile bioreactors that could operate under marine-like conditions so that organisms could be used and evaluated under physiological conditions similar to their habitats (Pomponi, 1999). Macroalgae and microalgae produce high-value compounds that cannot be obtained otherwise. Their vast majority and diversity in aqueous habitats constitutes a virtually inexhaustible reserve of natural substances and other by-products of commercial potential. The tendency and the challenge are to develop new products and test a new generation of natural products derived from these algae, in this way trying to replace synthetic for natural products.

Photosynthetic pigments. Among the bioactive products that microalgae produces, photosynthetic pigments might be some of the most exploited components in the food, drug, and cosmetic industries. These pigments, carotenoids (β-carotene, lutein, xanthophylls) and phycobiliproteins, have been gaining acceptance as natural colorants, replacing the synthetic ones since some of these synthetic pigments currently used are thought to be carcinogenics (Arad and Spharim, 1998). Carotenoid compounds have important nutritional and biological properties. β-carotene plays an important role in human nutrition due to its pro-vitamin A activity, also showing strong antioxidant activity, scavenging potentially harmful oxy radicals which are commonly associated with cancer and aging (Hejazi, *et al*., 2002). On the other hand, phycobiliproteins used for colorants in food (chewing gums, dairy products, ice sherbets, gellies, etc.) in Japan, Thailand and China, are also being used in cosmetics, such as lipsticks and eyeliners due to their intense unique pink color, especially the B-phycoerythrin (Bermejo-Roman et al., 2002).

These components have been exploited for years, however, there is still additional research needed to find parameters for maximum or optimum yields. There are several strains of microalgae that are currently being investigated for carotenoid production, *Dunaliella salina, Spirulina maxima, Clorella protothecoides* and *vulgaris, Haematococcus pluvialis*, among others (Canela *et al*., 2002; Choi *et al*., 2002; Li *et al*., 2002; Olmos-Soto *et al*., 2002; Shi *et al*., 2002). Not only its cellular production yield is important but also extraction yield. Different methodologies have been tested to maximize its selective extraction, such as supercritical fluid extraction with CO_2 (Canela *et al*., 2002) as well as regular nonpolar solvent exrtraction (Hejazi et al., 2002). A new methodology, the fermentative extraction of β-carotene using two phase systems and/or different light intensities without affecting the viability of the microalgae *Dunaliella salina* is being developed with promising results (Hejazi *et al*., 2002; Hejazi *et al*., 2003).

Pharmaceuticals. The potential for developing new drugs for cancer, AIDS, alzheimer's disease, among other illnesses has driven much of the research to search for new compounds from marine resources. Some early discoveries, such as manoalide and okadaic acid, referred to as pharmacological probes, showed potential to revolutionize our understanding of the underlying biochemistry disease due to their enzyme inhibiting activity (Fenical, 1997). Interest has increased markedly about the nutritional and pharmaceutical importance of polyunsaturated fatty acids especially ω3-fatty acids (ω3s) (Sahaidi and Wanasundara, 1998). The ω3s are essential components of cell membranes and of many cell-signaling systems and deficiencies are associated with defects in cellular function (Lewis *et al*., 1999). The potential increase in the demand for ω3s has led to increased in research into novel, sustainable sources of these compounds including microalgae (Lewis *et al*., 2000).

The ω3s of most interest are eicosanoids, while eicosapentanoic acid (20:5 ω3) and docosahexaenoic acid (22:6 ω3) are currently receiving much attention since they play a key role in the prevention and treatment of a wide range of human diseases and disorders. These include the prevention of heart disease by lowering cholesterol and low-density lipoproteins (LDL), lowering blood pressure and reducing clot formation (Nettleton 1995). They also have been found to inhibit cancer and tumor growth, improve the immune response and have a predominant effect on brain development. For these and other reasons they have been termed "essential" fatty acids (Lewis *et al*., 2000). Nowadays, the primary source of ω3s is fish oil, although microalgae is being considered as a potential alternative source of production. However, to be competitive with fish oil, algae culture optimization and scale-up bioreactors as well as ω3 extraction and isolation procedures need to be improved (Poisson and Ergan, 2001). Several marine microalgae have been proposed for commercial production of ω3 fatty acids such as *Porphyridium cruentum*, *Phaeodactylum tricornutum*, *Isochrysis galbana*, *Nannochloropsis oculata*, *Nannochloropsis sp*, *Monodus subterraneous*, *Crypthecodinium cohnii*, and *thraustochytrids* (Chini-Zittelli *et al*., 1999; Zou and Richmond, 1999; Acien-Fernandez, 2000; Poisson and Ergan, 2001; Sijtsma and de Swaaf, 2003). There exists the probability that other, more unusual PUFAs will be discovered in the time to come from this group of organisms (Lewis *et al*., 1999). The possibility that ω3s from these strains could enter the competitive market will solely depend on the ability to produce, refine, or enriched the oils to meet the market standards. Studies have also focused in essential vitamins for mammals produced by microalgae, such as vitamin B_{12}. Miyamoto et al. (2001) have suggested that vitamin B_{12} from unicellular Coccolithophorid algae might be bioavailable to humans.

A Sulphoglycolipidic fraction has been isolated from red alga *Porphyridium cruentum*. This fraction has the peculiar characteristic that strongly inhibits the production of superoxide anion, showing good antioxidant activity. It also inhibits the growth of human colon adenocarcinoma and to a lesser extent of human breast and prostate adenocarcinoma, and malignant melanoma, suggesting that it might have chemopreventive and chemotherapeutic potential (Berge *et al*., 2002). On the other hand macroalgae have also been studied because some novel terpenoid, phenolic, and carotenoid compounds have cytotoxic activity against human colon and lung carcinoma (Dorta *et al*., 2002; Yoo *et al*., 2002), as well as antibiotic, antifungal, antialgal toxic activity (Glombitza and Schmidt, 1999; Kimura and Maki, 2002; Wright *et al*., 2003).

Polysaccharides. Macroalgae are very well known to produce polysaccharides of commercial significance for the food and pharmaceutical industry. These polysaccharides are agar, carrageenans from red algae, and alginate from brown seaweeds (Powers, 1990). These gel-forming polysaccharides have a wide variety of applications in different fields; they can function as thickeners, emulsifiers, and stabilizers in food as well as in the paint and photographic industries having great functionality over a broad range of conditions. They are also used in studies of fundamental techniques in biochemistry, molecular, and cell biology and can be also used by the pharmaceutical industry as dietary fiber (Arad and Spharim, 1998). Interestingly, carrageenans appear to have good anti-HIV-1 (human immunodeficiency virus) activities and low anticoagulant properties that may justify clinical trials (Vlieghe, *et al*., 2002).

Other compounds and uses. Currently, the demand for new biomaterials and bioproducts such as silica is increasing. There is a photosynthesizing microalgae of the family Bacillariophyceae (diatoms), (e.g. *Cyclotella cryptica*) which produce silica exoskeletons with detailed species-specific morphological features with a potential use in specific industrial or technological processes. They create high surface area that is interesting with respect to new developments in synthesis of silica-based materials (Vrieling *et al*., 1999; Csögör *et al*., 1999).

Currently, there have been studies showing the potential biocide use of sesquiterpenoids esters from the green alga *Caulerpa prolifera* and halogenated furanones from the red alga *Delisea pulchura* as antifouling compound for use in marine paints to replace the highly toxic organotin tributyltin oxide (TBTO), compound that for years has received attention for its toxicity to aquatic life (de Nys and Steinberg, 2002; Smyrniotopoulos *et al*, 2003). Among other compounds currently under research for their application as natural sunscreens are the photoprotective UV-absorbing compounds mycosporine-like amino acids (MAAs) and scytonemin from macroalgae (as well as from other marine organisms). These compounds act to protect the organisms from harmful effects of UV light, thus having the potential for the development of novel UV blockers for human use (Hoyer *et al*., 2001; de Nys and Steinberg, 2002).

Algae can also be used in environmental applications such as in the bioremediation of contaminated marine ecosystems as well as wastewater. They show a high capacity for assimilation of toxic heavy metals such as lead, copper, nickel, cadmium, selenium, and tin among others, with rates of accumulation being metal specific. They can remove inorganic nitrogen sources from aquaculture effluents and have a high probability of being used for detoxification of organic pollutants such as herbicides and pesticides (Bird *et al*., 1998; Klimmek *et al*., 2001; Lin *et al*., 2002).

Marine sponges

As with algae, sponges are a rich source of new bioactive natural products waiting to be discovered. Approximately one-third of all marine natural products known so far have been isolated from sponges. This makes sponges the most popular source for research in this area (Wang, *et al*., 2003). They have been recognized as the most prolific sources of chemically diverse classes of compounds with a variety of biological and pharmacological activities including antimicrobial properties with potential to become effective drugs for therapeutic use (Kuniyoshi and Higa, 2001). Kuniyoshi and Higa (2001) report that in the decade (1990-1999) there were 590 antimicrobial compounds reported and grouped as terpenoids, steroids, nonterpenoids, and nitrogenous compounds. Besides their antimicrobial effect, some of these compounds show a multifunctional biological activity as shown below. Due to their abundance and lack of space, just a few compounds derived from marine sponges will be described.

Pharmaceuticals. A large number of compounds have been isolated from marine sponges. They have attracted considerable attention because of their unique structures, rich physiological properties, and activities against a variety of human diseases (Li et al., 2003). Many of these compounds are thought to be produced by symbiotic bacteria that live within the sponge itself (Faulkner et al. 2000). By far the largest number of sponge bioactive compounds contains at least one nitrogen atom. Among these, exist a rich source of unusual peptides possessing such biological activities as cytotoxic, enzyme inhibitory, antifungal, and anti-HIV. Some of these novel compounds found in sponges are: depsipeptides nagahamide A from *Theonella swinhoei* with antibacterial activity (Okada *et al*., 2002) and halipeptins A and B with a very potent anti-inflammatory activity (Randazzo et al., 2001), some cyclic heptapeptides such as leucamide and phakellistatin 13 that have shown to be moderately active against several tumor cell lines (Kehraus *et al*., 2002; Li *et al*., 2003) and some tetramic acids such as melophlins (Wang *et al*., 2003). In addition some steroids and lipids (mololipids) have pharmacological properties with strong bioactivity against bacteria, virus and cancer cells (Ross *et al*., 2000; Gallimore *et al*., 2001). However, according to Müller (2003), of the hundreds of compounds discovered with biological activity, only a few have undergone clinical trials due to the limited amounts of starting material. Efforts are underway to cultivate these organisms either at sea or in bioreactors to allow for sustainable production.

Sponges also produce compounds that affect the attachment of bacteria and/or fungus, thereby inhibiting its colonization and preventing subsequent steps in the fouling process. Extracts from several genus (Ircinia) (Tsoukatou *et al*., 2002) and species (*Agelas conifera*, *Ailochroia crassa*, *Chondrilla nucula*, among others) have shown antifouling properties, inhibiting the attachment of bacteria (Kelly *et al*., 2003). Other marine invertebrates such as bryozoans, small sessile animals, have been studied for their production of bioactive metabolites (Haygood et al., 1999). *B. neritina* is a bryozoan and the source of bryostatin a cytotoxin compound that has potential as a cancer drug, and is in clinical trials for leukemias, lymphomas, melanoma, and solid tumors. A major constraint in developing clinical trials is obtaining sufficient quantities of the compound. Work is underway to develop model systems of production identifying specific symbiotic bacteria that produce the bryostatins with or without the host bryozoan.

Marine microorganisms

The unique environment of the oceans has proven to be a veritable breeding ground for microorganisms that produce unusual metabolic compounds in response to the extreme conditions of their environment. It is estimated that less than 10% of marine microorganisms have been classified due to the difficulty of culturing them in the laboratory. Many of these organisms live in extreme environmental conditions compared to terrestrial microorganism. The term extremophiles refers to organism that can tolerate or thrive in environments that are outside the parameters of most living things (www.micro.unsw.edu.au/rick/extremeophiles). Most extremophiles are microorganisms and many live in the marine environment. These organisms tolerate saline conditions, can grow at below freezing temperatures as well as extreme high temperatures (thermal vents) and live in conditions of high pressure. Enzymes that function under extreme conditions of pressure, pH and temperatures have potential for developing new food processing systems in the future (Fujiwara, 2002). These marine organisms possess cellular processes that are optimally adapted for growth in the extreme environment in which they evolve (Cavicchioli *et al*., 2002). They possess unique sets of enzymes with exceptional properties that can be advantageous not only for the food and biotechnology industries but can also be used in unique bioprocess operations. Most of these extremophiles belong to the third domain of life, archaea which differ from bacteria and multi-cellular eukaryotes (Fujiwara, 2002). The biotechnology potential increases with the discovery on new organisms and pathways that produce new products with potential drug-related applications. On the more practical side, the extremophiles produce unique enzymes that have application in several industries as shown in Table 2. The discovery of thermal vents in the deep oceans and the the new organisms that survive in this extreme environment is but one example of potential new enzymes and metabolic pathways that will open new doors for marine biotechnology.

Table 2. Applications of enzymes and compounds from extremophiles, (Fujiwara, 2002)

Microorganisms	Enzyme, endogenous compounds	Applications, products
Thermophiles(50-110°C)	Amylases	Glucose, fructose for sweeteners
	Xylanases	Paper blanching
	Proteases	Amino acid production, food processing, detergents
	DNA polymerase	Biotechnology
Psycrophiles (5-20 °C)	Neutral proteases	Cheese maturation, dairy
	Lipases	Detergents
	Polyunsaturated fatty acids	Pharmaceuticals
	Ice-protein	Artificial snow
Acidophiles (pH<2)	Sulphur oxidation	Desulphurisation of coal
Alkaliphiles (pH>9)	Lipases	Detergents
	Cyclodextrins	Stabilization of volatile substances
	Antibiotics	Pharmaceuticals
Halophiles (3-20% salt)	Carotene	Food coloring
	Glycerol	Pharmaceuticals
	Compatible solutes	Pharmaceuticals
	Membranes	Surfactants for pharmaceuticals

Marine Enzymes and Waste Utilization

There are several excellent reviews on the utilization of enzymes from aquatic organisms (Shahidi and Kamil 2001; Gilberg, Simpson and Haard, 2000; Haard, 1998; Vilhelmsson, 1997; Haard, 1992). Enzyme extraction and utilization from seafood wastes include enzymes extracted from both solid and liquid waste streams that were often previously discarded. These include extracellular gastric enzymes from aquatic vertebrates and invertebrates, intestinal proteases, cellular proteases, lipases, phosphatases and others. There has been considerable research in this area because of the unique environments of the organisms and unusual specificity of several enzymes as well as the environmental and ethical concerns surrounding waste discards. Several of these enzymes are salt tolerant and tend to have higher activities, especially at colder temperatures (Cavicchioli et al., 2002). Until recently there were few successes in incorporating marine enzymes in food processing applications. This is slowly changing and there are more applications being commercialized. Fish fermentation to produce products such as cured herring fish sauces is moving in the direction of a more controlled fermentation with specific enzymes. Maatjes is a fermented product using the natural viscera enzymes in herring. Several efforts have been made to describe the enzymatic reaction and mimic the end result (Olsen and Skara, 1997; Nielsen and Borrensen, 1997; Nunes et al., 1997). More success has been made with using enzymes hasten the process and quality of fish sauce production. Researchers (Tungkawachara et al, 2003; Chaveesuk et al, 1993) have shown the efficiency of using specific enzymes for the production of fish sauce and producing an acceptable product. . Although enzymes from plant and microbial sources are commonly used for the production of fish protein hydrolysates, research with marine enzymes has shown comparable production rates (Benjakul and Morrissey, 1997). Shahidi and his coworkers have produced fish protein hydrolysates using mixtures of extracted and endogenous enzymes in both capelin and seal meat (Shahidi et al. 1995; Shahidi and Synowiecki, 1997). The enzymatic removal of fish skin from certain species has proven advantageous as an alternative method for mechanical deskinning (Tschersich and Choudhury, 1998; Kim et al, 1993). The use of proteases for removal of squid skin is a standard process, although non-marine proteases are often used. Several researchers have shown that marine enzymes can be used and often leaves a product that has several advantages over papain or ficin proteases (Strom and Ra, 1991; 1993; Wray, 1988) . Research has also shown the efficacy for higher yields in fish caviar for salmon and lumpfish and the development of pearl essence (Raa 1997).

Although many of the marine enzyme technologies are in the initial phases, there have been notable success in biochemistry/biotechnology field. The isolation and purification of shrimp alkaline phosphatase (SAP) from cold water shrimp (*Pandalus borealis*) has led to the use of this compound in gene splicing with plasmid and bacteriophage vectors in most biotechnology laboratories (Olsen et al., 1991; Sambrook and Russell., 2001). SAP has the advantage over other alkaline phosphatase in that it can be denatured at lower temperatures (65C for 15 min) and not denature DNA materials. The enzyme is recovered and purified from the shrimp processing waste water and is a highly sought after chemical. This success has created other potential opportunities with waste enzymes and Biotec, ASA in Norway also lists cod uracil-DNA glycosylase and other enzymes for use in biotechnology and food processing fields.

There are several other compounds that can be recovered from waste and have potential value. Chitin and chitosan are recovered on a commercial basis from crustacean shell-waste and are active ingredients for a number of products (Ludlow, 2001). These include the pharmaceutical industry where chitosan is incorporated into wound-healing pads that have both hemo-static and antibacterial activity. In the nutraceutical industry chitosan is being touted a a fat-reducing agent for those that are concerned of lipids in their diet. There are numerous applications of chitosan in the fields of cosmetics, fiber and textiles, agriculture, paper and waste industries. However, the costs of application often does not meet the manufacturing costs and therefore the majority of the

utilization is at the high value product end. Research is needed to produce chitosan as a high-volume low-cost product that allow its use in other fields. There is also great interest in developing new antibiotics from marine sources and several peptides have shown promise. Antibacterial peptides have been isolated from winter flounder (Saint et al, 2002), horseshoe crabs (Tamamura, 1993), and shrimp (Bachere et al., 2000). Pleurocidin, a 25 cationic peptide from the mucous of winter flounder was found to be affective in combating disease in coho salmon when inoculated with *Vibrio anguillarum* (Jia et al., 2000). Protamines which are extracted from fish milt have proven to have antibacterial activity in several food pathogens including L*steria monocytigenes* and *E. coli* (Hansen and Gill, 2000).

The question remains about the economic feasibility of recovery of specific compounds from waste. Although there have been numerous technological advances in the recovery of specific compounds from fish waste, there is more research that needs to be done (An and Visessanguan, 2000). Furthermore, there is competition for the waste uses. Many fish processing operations have utilized solid wastes for traditional products such as fish meal, fertilizers, silage and more recently hydrolysates. Many of these end uses have stable markets and there is a known capital investment as well as operational costs. Extraction of specific enzymes from fish wastes requires investment in technologies that often have high costs and varying efficiencies. For example, the use of specific enzymes from fish wastes for the removal of skin from squid will yield a product of good quality and reasonably costs. However, much of the industry currently uses ficin and papain which are low costs enzymes that perform well under most conditions. Therefore it is difficult to make inroads into marketplace unless there is a cost advantage to the end-user. DeWitt and Morrissey (2002) showed that it was feasible to concentrate cathepsin L from Pacific whiting waste water. However, they were not able to do so in concentrations sufficient to compete with other commercial enzymes. The advent of gene technology and the production of specific compounds through the use of microbial organisms also causing some degree of risk in heavily investing in recovery systems. As this technology continues to develop over the next decade, enzymes or proteins with unique characteristics from marine organisms may be duplicated and produced more economically that specific recovery from waste materials or directly from the organism itself. Even in the case of shrimp alkaline phosphatase there is active research to transfer the SAP gene to bacteria to produce greater quantities of this valuable enzyme.

The recovery of specific compounds in waste streams does little to reduce waste and may often create new waste streams that are an environmental headache and too costly to the company. For example, the recovery of chitosan from shrimp waste represents a yield of 3% (wet weight) from the raw waste material and the use of caustic chemicals in chitosan production is mitigated at a high cost. The best way to reduce waste is to develop new methods to increase yields or develop primary products for what were formerly waste streams. The surimi industry is a case in point. During the initial period of the U. S. surimi industry in the 1980s, yields were often 15-17% of the raw material. For every 100 pounds of pollock harvested, 15 kg of surimi was produced. The use of high speed centrifuges for protein recovery in waste water streams helped to raise the recovery over 20%. With the repassage of the Magnussen Act in 1995 with a focus on full utilization, there were incentives in the industry to further increase utilization. Markets were developed for specialty products such as fish stomachs and milt. New filleting and cutting machines were developed to extract a higher percentage of the flesh from the fish before mincing. Special attention was paid to washing conditions (time/temperature) to minimize protein loss in the dewatering step. These incremental steps improved surimi yields to greater than 27% and opened new markets for by-products for the surimi process.

One of the more interesting technological advances for protein recovery from fish flesh is the pH extraction method developed by Hultin and Kelleher (1999, 2000). This method uses the solubility of fish protein at pH extremes (both acid and alkaline) as a method of efficient protein recovery. Both myofibrillar and sacroplasmic protein are highly soluble at acid pH (~3.0) and alkaline pH (~10.5). This allows the efficient separation of protein from non-protein material such as lipids, membranes, skin, bones, etc. The process is as follows: fish minus the heads and viscera are homogenized and the proteins solubilized either by acid or alkaline (Undeland et al., 2002). Centrifugation separates impurities such as skin and bones while the lipid layer floats to the top. The pH is then adjusted to the fish protein isoelectric point of 5.3-5.5 whereby the fish proteins precipitate out of solution and are recovered by centrifugation. Close to 100% of the myofibrillar protein is recovered as well as the majority of the sacroplasmic protein. Yields have been reported as high as 40% for some species and there is little protein that is lost in the waste water thereby decreasing disposal costs. Protein functionality also remains high even though there is some denaturation at the pH extremes. Several pilot-scale trials are now underway to determine the feasibility of this process at a commercial level (Hultin, personal communication).

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L 69. UTILIZATION OF FISH BY-PRODUCTS IN ICELAND

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Abstract

Fisheries are the single most important industry in Iceland and it will continue to play an important role in the economy of Iceland for a long time to come. In 2001, the total catch was around 2 million tons, accounting for 62% of country's merchandise exports. The living marine resources are however limited and it is important to utilize these resources in a sustainable way, as well as to maximize their value by producing high-priced products from the raw material which is currently being used for fish meal or simply discarded. For example, today all cod heads from land-based processing plants are being utilized and lately the freezing trawlers have begun freezing them onboard for processing onshore. Fortunately, most of the by-products are no longer regarded as waste but are used as raw material for fish processing like roe, liver, mince, viscera etc.

The by-products from salting, freezing, canning, fresh fish and other processes have different qualities and potentials. Therefore, quality management is important and new technologies are emerging that will allow a new range of products to be made from by-products which will e.g. benefit the pharmaceutical, cosmetics and food industries worldwide.

Introduction

The living marine resources in Icelandic waters are the most important natural resources in the country. In 2001, the total catch was around 2 million tons, accounting for 62% of the value of exported products and around 48% of the foreign currency earnings that year. The most important fish species in Icelandic waters belong to the gadoids; cod, haddock, pollock and blue whiting. Other important species are ocean perch, Greenland halibut, herring, capelin and shellfish like shrimp, lobster and scallop, (Figure 1).

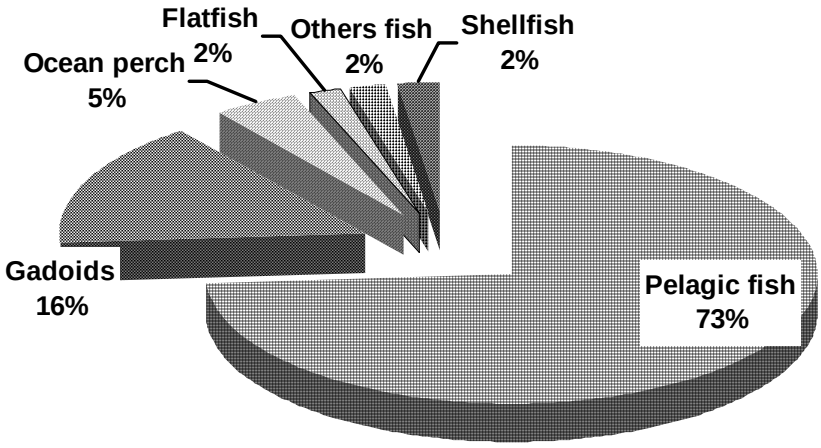

Figure 1. Catch of Icelandic vessels by fish spices fishing for 2001, the total catch was 1,941,905 tons.

Fish meal and oil constitute the bulk of the volume of products from fisheries in Iceland or 63% of total, but their value is far less or only about 14% of the total value of exported seafood products (Figure 2).

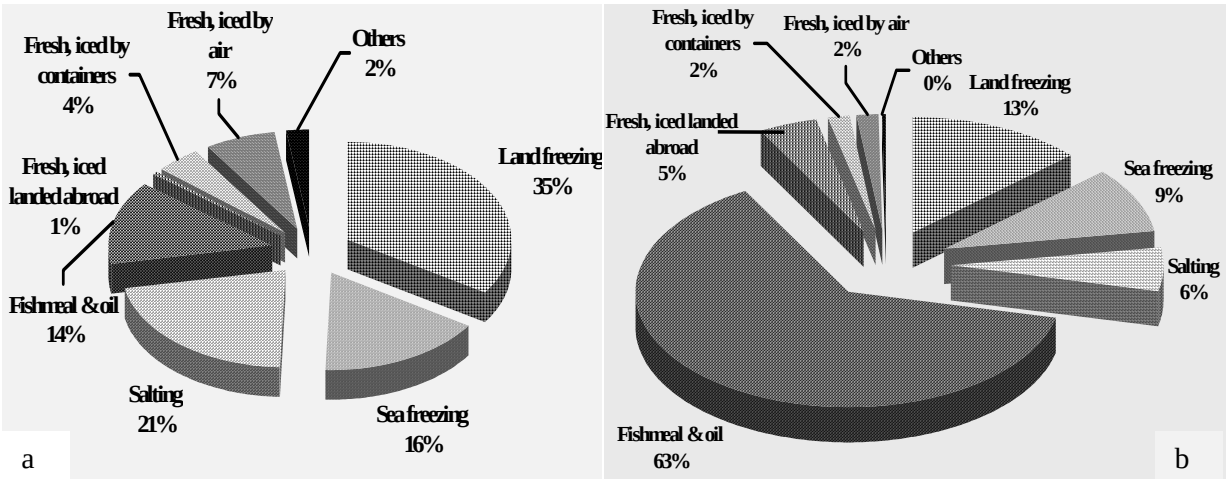

Figure 2. The main Icelandic fishery products area 2001. (a: Export value, b: Quantity of export).

The Icelandic fishing fleet and the processing plants are highly mechanised as can be seen by the fact that only about 10% of the work force is employed in fishing and fish processing. Icelanders, like may other nations, have realised that their fish resources are limited and that a collapse of any of the major stocks would be economically disastrous. In 1984 fixed quotas for each vessel were introduced in order to control exploitation of the fish stocks (Valdimarsson 1990). The main objective of the quota legislation was to prevent over fishing and to encourage responsible handling of all catches and exploitation of under-utilised marine life. There is no doubt that the quota system has had a major effect in changing the attitude toward full utilisation of the catches. The fishermen and the processing industries are becoming more aware of the possibilities of making marketable products from raw materials that currently are either used for fish meal or simply discarded. Through R&D, publicly funded institutions assist the industry to increase utilization of seafood.

During the last decade, the annual fish catch has stabilized at about 1.5-2 million tons, and according to fish biologists, no further growth is expected in the near future. Hence, optimal utilization of fishery ”by-products” is becoming increasingly important to provide more marine fish raw material for various industrial purposes.

Utilization of Fish ”By-products”

The discussion on ”by-products” from ground fish will be divided into three categories: In the first utilizing the viscera, in the second utilizing the frames, cut offs etc. and finally the utilization of heads, which represent the main volume and value of the export of ”by-products” from Icelandic fisheries (Figure 3), (Arason S., 2001, Arason S., 2002)

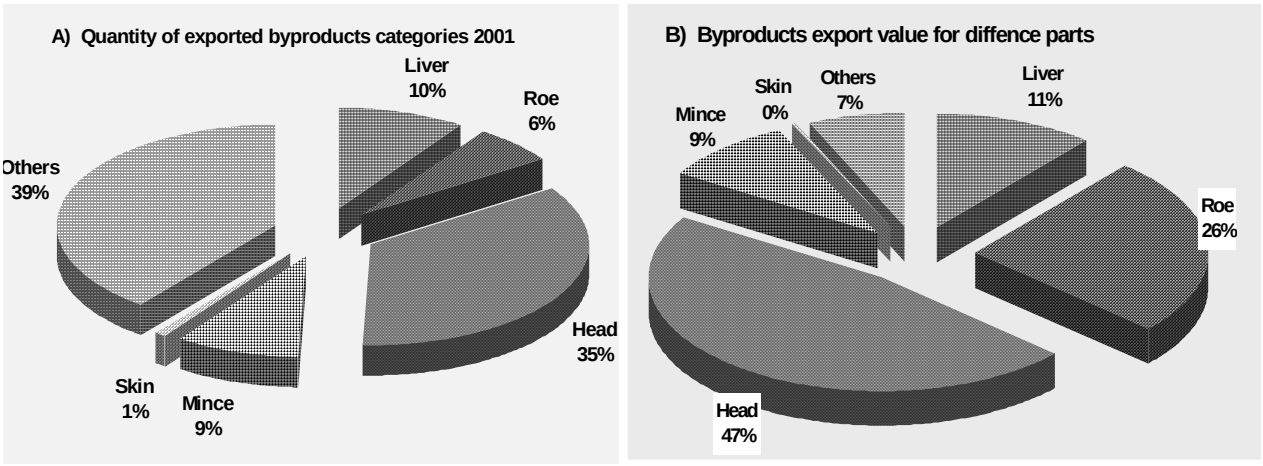

Figure 3. Export of by-products from groundfish processing in Iceland 2001. A: Quantity of export byproducts 2001 was 45,543 tons. B) Export value for 2001 was 73.5 millions $.

The Future

One thing that all agree on is that there is not an endless amount of fish in the sea and therefore we need to manage our fisheries better than in the past. There are many ways, which are possible to optimize fisheries management. It is, however, unlikely, that we can increase the catch of wild fish but we can increase fish farming. Furthermore, there are many ways to increase the value of our catches.

Annual discard from the world fisheries has been estimated to be approx. 25% of the total catch. Utilisation is driven by economic factors. The conditions onboard the vessels are currently not optimised for a cost effective utilisation. Therefore, the main challenge will be to build knowledge on the market potential of the different by-products and which processes, for practical reasons as well as preservation, should be done onboard the vessel and which on shore.

Increasing the proportion of the catch intended for human consumption and other value added products (pharmaceuticals, feed ingredients etc.) would increase the profitability and reduce the amount of "waste".
In order to achieve this it is necessary to:

- ✓ Develop systems to sort and handle the by-products on board
- ✓ Find safe and cost-effective preservation methods
- ✓ Improve logistics to get the by-products from the vessels to the processing plants

In order to upgrade the by-products, more knowledge is needed on the chemical composition, including seasonal variation, of the fishing stocks. This is necessary in order to find effective and safe preservation and storage procedures and to find biomolecules with possible application in the food, feed and pharmaceutical industries (Figure 4).

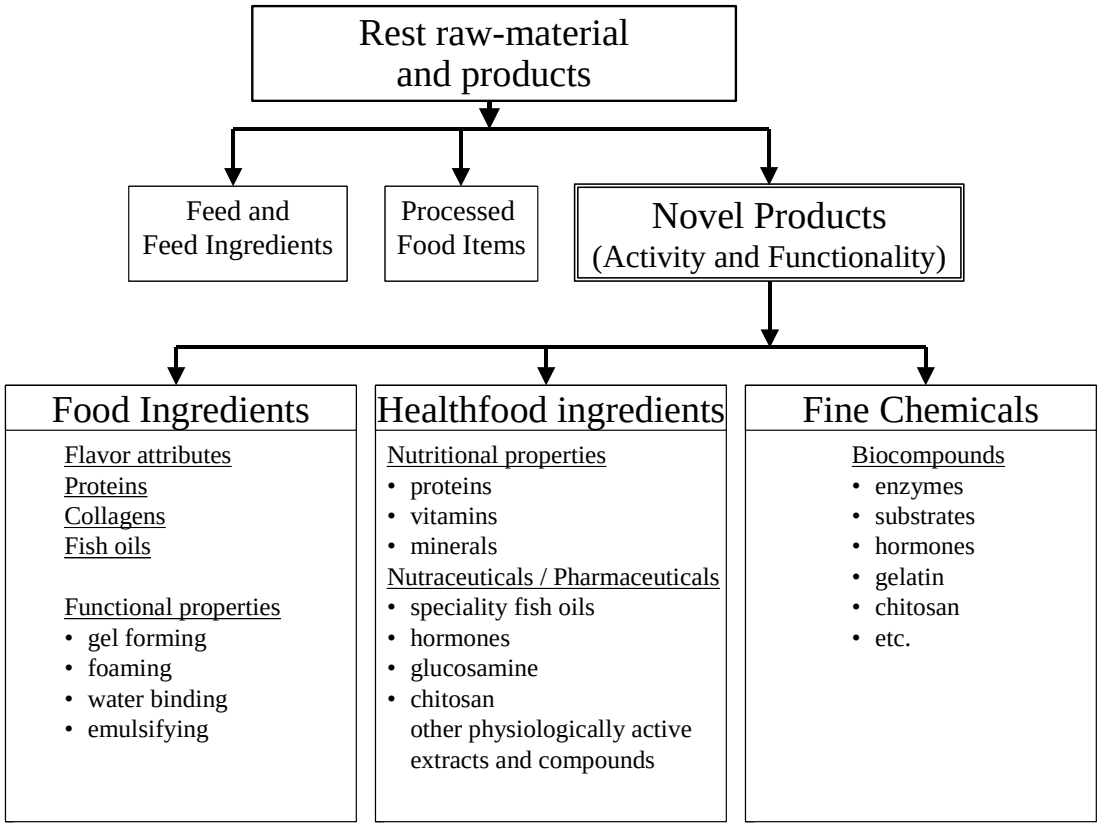

Figure 4. There are many interesting possibilities for by-products from fish.

More knowledge is also needed on processing methods to extract the interesting fractions/biomolecules. Finally, there is a need to study the market for these compounds. By-products contain components with applications in food, healthcare products, pharmaceuticals and cosmetics. Screening of the valuable components and an evaluation of the market situation for each component are needed.

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L 70. RECENT DEVELOPMENTS IN DEACETYLATION OF CHITIN, AND POSSIBLE APPLICATIONS IN FOOD FORMULATIONS.

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Abstract

Chitin is the second most abundant biopolymer in nature after cellulose. It is the main constituent of the protective shell in insects and crustaceans, like shrimp, lobster and crab. The biopolymer is composed of (1→4)-2-acetamido-2-deoxy-β-D-glucan, which is bio-degradable but not water-soluble. The acetyl group connected to an amine group in the C_2 position on the glucan ring, may be removed by enzymatic or chemical hydrolysis in caustic soda at elevated temperatures, producing a deacetylated form exposing free amino groups at some of the C_2 positions. When the fraction of acetylated amine groups (F_A) is lower than 0.35-0.40, the co-polymer of (1→4)-2-amino-2-deoxy-β-D-glucan and (1→4)-2-acetamido-2-deoxy-β-D-glucan is referred to as chitosan. Chitosan may be washed with water and dried into flakes or milled into powdered chitosan. The flakes or powder may be dissolved in acid to form a 2-3% solution and titrated with alkali to form precipitate of microcrystalline chitosan (MCCh), a highly viscous suspension with high water holding capacity.

Chitosan has been used as a dietary supplement due to its effective lipid binding and hypocholesterolemic properties. There are indications from recent reports that low molecular forms of the chitosan family may possess antimicrobial activity, as well as antioxidant activities, probably through an iron scavenging function. Furthermore chitosan has been reported to be effective as a film-forming agent. Many other potential medical and pharmaceutical applications have been proposed. All of the reported properties of chitin and chitosan are highly dependent upon polymer composition and environmental pH. The co-polymer becomes positively charged at pH below the isoelectric point of the free amine group (approx. pH 6.5), giving a highly bitter and dry aftertaste, which limits the applicability of chitosan in some food formulations.

Chitin may be deacetylated to a lesser extend than is common for conventional chitosan (F_A between 0.50 and 0.70) and it has recently been demonstrated that the sequence of deacetylation may be controlled (homogenous versus heterogenous distribution of the N-acetyl groups). These chitin materials have properties, which differ from both chitin and conventional chitosan varieties and the compound is soluble over a wide pH range. The paper will review recent developments in characterization, identification and potential applications of such minimally deacetylated chitin in food formulations.

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L 71. BITTERNESS IN FISH PROTEIN HYDROLYSATES: ORIGIN AND METHODS FOR REMOVAL

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Abstract

Enzymatic hydrolysis has several distinct advantages over other processing methods for recovering the protein from under utilized fish biomass. However, the hydrolysis of proteins from fish and fish by-products may create problems due to the formation of short chain peptides, thus causing the development of bitter taste in the product, this restricts the practical uses of these hydrolysates. The presence of bile in the raw material from fish by-products may also influence the development of bitterness in fish protein hydrolysates (FPH).

Three different methods were used to eliminate or reduce bitterness from FPHs after enzymatic hydrolysis: 1) secondary treatment with endopeptidases (Flavourzyme), 2) treatment with butanol and 3) treatment with cholestyramine resin. Flavourzyme is used to reduce the bitterness due to hydrophobic amino acids in peptides. Butanol is an alcohol not soluble in water, which permits the extraction of hydrophobic amino acids, peptides and other hydrophobic compounds. The cholestyramine resin is known as a drug and a chromatography absorbent to remove bile acids and related compounds found in bile. The use of these compounds separately or in combination showed promising results: the bitter taste from FPH was reduced to levels undetectable by our sensor panel.

Introduction

Shortly after the Second World War, Canadian researchers developed methods for the enzymatic preparation of protein hydrolysates from fish meat (Tarr, 1948). Today it is well known that many of the fish flavour, fish soup and fish paste products available on the market are prepared by enzymatic hydrolysis (Shoji, 1990). One of the shortcomings for the wide application of the protein hydrolysates is their bitter taste. The chain length of the peptides of the hydrolysate is of special interest in relation to the organoleptic and functional characteristics, because properties like solubility, emulsifying capacity and bitterness depend, at least in part, on molecular size (Mohr, 1980). According to this hypothesis, bitterness is related to the average hydrophobicity of the peptide. Moreover, the taste of products obtained by proteolytic modification of proteins depends on their degree of hydrolysis (Shahidi, 1994). Low bitterness could usually be ascertained by restricting the DH to values of 3-5% (Adler–Nissen, 1984). Alternatively, a high DH may reduce the bitterness: a complete hydrolysis to free amino acids decreases the bitterness, because hydrophobic peptides are considerably more bitter than the corresponding mixture of free amino acids (Belitz and Wieser, 1976). A risk of bitterness increases when the % of DH is between 4 and 40%.

The bitterness reaches a maximum when the hydrophobic amino acid is blocked at both ends through a peptide bond. The bitterness is lower when hydrophobic amino acids are in the C- or N- terminal position and lowest when the amino acids are free (Liaset et al., 2002). Bacterial endoproteases have specificity towards hydrophobic amino acids. By applying Flavourzyme with its combination of exopeptidase and endoprotease activity, the risk of bitterness is minimised (information sheet, Novozymes A/S, Denmark).

Presence of bile acids in fish biomass may form unwanted bitter taste in the end product. Bile is only a very small part of fish intestines. When raw material contains bile, bitter taste after hydrolysis can also come from bitter peptides or bile acids. The bile acids are the end products of cholesterol utilisation, synthesised in the liver. Kahlon and Woodruff (2002) used soy protein, pinto beans, black beans and wheat gluten to bind bile acids in vitro, black beans had the highest binding ability. Cholestyramine resin (a bile acid binding and cholesterol lowering drug) can be also used for the binding bile acids and removal bitter taste from hydrolysates.

Bitter taste of autolytic extracts was found to be enhanced by shaking treatment because of the accelerated lipid oxidation (Liu *et al*., 2000).

However, despite the problem with bitterness of fish protein hydrolysate, little research has been conducted on protein hydrolysates (FPH) especially on fish hydrolysates made with enzymes for human food applications (Kristinsson and Rasco, 2000).

The objective of this work was to determine a) whether presence of bile in the raw material increases the bitterness in the FPH. b) Bitter taste of fish protein hydrolysates is caused by bitter peptides or by components from the bile? Three different methods were used to eliminate or reduce bitterness from FPHs after enzymatic hydrolysis: 1) secondary treatment with endopeptidases (Flavourzyme), 2) treatment with butanol and 3) treatment with cholestyramine resin.

Materials and Methods

Farmed cod (*Gadus morhua)* were used for the experiments. Intestines with and without gall bladder were used. Samples were mixed with the equal amount of distilled water and hydrolysed in a closed glass vessel at 50ºC for 60 min after adding 0.1% enzyme (Flavourzyme 500L or Alcalase 2.4L, Novozymes A/S, Denmark) followed by inactivation for 5 min at 90ºC in microwave oven. The secondary treatments were performed with 1) endopeptidases (Flavourzyme), 2) butanol and 3) cholestyramine resin (Sigma-Aldrich). Mixtures after hydrolysis and secondary treatment were centrifuged, liquid part was separated, freeze-dried and 1% water solution was presented for taste evaluation.

Ash content was estimated by charring in a crucible at 600^{o}C until the ash had a white appearance (AOAC, 1990). CHN-S/N elemental analyser was used to determine the total N and the crude protein was estimated by multiplying total N by the factor 6.25. The extraction of total lipids from the samples was performed according to the method of Bligh and Dyer (1959). The degree of hydrolysis was evaluated as the proportion (%) of α-amino nitrogen with respect to the total N in the sample (Taylor, 1957). Amount of free amino acids was determined by reversed phase HPLC by pre-column fluorescence derivatization with o-phthaldialdehyde, using a NovaPak C18 cartridge (Waters, Milford, MA, USA) and the method of Lindroth and Mopper (1979) as modified by Flynn (1988). Statistical programs Guidelines (CAMO ASA, Oslo, Norway) and Microsoft Excel were employed for data processing and statistical analysis. Significance was determined at the 95% level.

Results

It was observed that powders obtained from the samples with presence of gall bladder had significantly higher bitter taste then powders without bile independent of the type of enzyme used. Initial treatment with Alcalase resulted in more bitter samples than hydrolysis with Flavourzyme.

The samples without secondary treatment and samples after Flavourzyme secondary treatment did not show significant difference in the bitterness, only in increased yield.

Cholestyramine resin was most efficient in the removal of bitterness and yielded powders with the best colour. Butanol also reduced bitterness of the powders but powders had dirty green colour and tasted of butanol. All chemical removal of bitter taste improved the taste but reduced the yield of FPH.

The DH of samples was higher than 40% but was still bitter. This bitterness was removed only by chemical treatment.

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L 72. VOLATILE FLAVOR COMPOUNDS IN SEAFOOD FLAVORANTS

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Abstract

Seafood flavors using residue products e.g. shrimp shells, fish cut-off, fish skin and bones can be produced by enzymatic methods. Using controlled proteolytic hydrolysis coupled with other processing methods, Primex ehf. has been able to establish high quality products such as Shrimp, Cod and Pollack flavors. The company has been able to drive their processes in the direction of enhancing some of the species characteristic compounds, and toward a more balanced flavor profile.

The aim of this study was to characterize the flavor compounds in enzymatically produced flavorants by Primex ehf. The flavorants were produced from e.g. cod, pollack, shrimp shell, scallop and whey. The outcome is a basis for a database to be used for the development of recipes for marine flavors by Primex ehf.

Flavor compounds were studied by using gas chromatography analysis (GC/Olfactometry and GC/Mass Spectrometry) for the screening of volatile compounds. Eight types of product samples together with peeled and unpeeled shrimp were studied. The most characteristic volatiles in the flavorants were methional and *cis*-4-heptenal, giving a potato-like odor, 2-acetyl-1-pyrroline giving a popcorn-like odor and degradation products of fatty acids (e.g. 1-octen-3-ol, mushroom) that give a mild, characteristic seafood-undertone. Many of these compounds are produced during enzymatic and thermal processing of the flavors. Characteristic popcorn-like odor in shrimp was found to be in the shell and was only identified in unpeeled shrimp.

Materials and Methods

Samples. Volatile flavor compounds of eight types of flavor samples together with peeled and unpeeled shrimp were analyzed using gas chromatography/olfactometry (GC/O) and gas chromatography/mass spectrometry (GC/MS) together with sensory analysis. The flavors were produced from fish cut-off, shrimp shells, scallop and whey using different kind of enzymes.

Sampling and gas chromatography techniques. Prior to GC/O and GC/MS analysis flavor samples were collected by a purge-and-trap sampling on Tenax traps (Olafsdottir and others 1985). Volatile compounds were separated on a fused silica capillary column, DB-5ms (J&W Scientific, Folsom, CA) using helium as a carrier gas. GC/MS measurements to identify volatiles based on their mass spectra were performed on a HP G1800C GCD (Hewlett-Packard, Palo Alto, CA). GC/O measurements to identify compounds based on their characteristic odor were performed on a HP 5890 gas chromatograph (Hewlett-Packard, Palo Alto, CA). Two persons describing the odor sniffed the effluent. Intensity of each odor was determined using an intensity scale from 0 - 5, 0: not present; 5: very strong.

Identification of the volatiles was done by matching retention indices (RI) of ethyl esters and mass spectra of samples with authentic standards (Sigma-Aldrich and Merck). Tentative identifications were based on standard MS library data (Hewlett-Packard Co, 1997 and manually checked against literature sources and the database Flavornet (Acree and Arn 1997).

Results and Discussion

A total of 38 volatile compounds were identified in the seafood flavorants including 20 aldehydes, 10 ketones, 2 alcohols and 3 sulfur-containing compounds. The most characteristic volatiles in the flavorants are listed in Table 1. Among them are methional and *cis*-4-heptenal giving a potato-like odor. Methional may be formed via Strecker degradation of the amino acid methionine (Baek and Cadwallader 1997). It has been identified as an important contributor of seafood flavor in various crustaceans and flavor concentrates (Baek and Cadwallader 1997; Chung and others 1995; Kim and others 2000). Cis-4-heptenal, a lipid-derived compound can also contribute to potato-like odor when detected in low concentration (Josephson and Lindsay 1987; Chung and Cadwallader 1994). This characteristic potato-like odor was not identified in peeled or unpeeled shrimp.

Table 1. The most characteristic volatile compounds identified in seafood flavorants by GC/MS and GC/O.

Compound	Identifiaction[a]	GC/MS rt (min)	RI[b]	Odor
cis-4-heptenal	MS, 1, 2	8,9	494	rancid, potato-like
methional	MS, 1, 2	9,1	502	potato-like
2-acetyl-1-pyrroline	3	10,6	536	popcorn-like
1-octen-3-ol	MS, 1, 2	12,3	576	mushroom
trans-2, *cis*-6-nonadienal	MS, 1, 2	19,1	760	cucumber

[a]Identification means: MS, mass spectra; 1, authentic standards; 2, odor identification; 3, odor identification and RI references

[b]Calculated ethyl ester retention index on DB-5ms capillary column

2-acetyl-1-pyrroline, giving a popcorn-like odor, was also detected as a key aroma compound. It is thermally generated and may be formed via the Maillard reaction (Baek and Cadwallader 1997). Characteristic popcorn-like odor in shrimp was found to be in the shell and was only identified in unpeeled shrimp. Lipid derived volatiles like 1-octen-3-ol and 2,6-nonadienal, giving mushroom- and cucumber-like odor, respectively, gave a mild characteristic seafood-undertone.

Conclusion

The GC/O and GC/MS results indicate that the key aroma compounds detected in the seafood flavorants are a combination of aldehydes and lipid derived carbonyl compounds produced during enzymatic and thermal processing of the flavors.

Acknowledgments

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L 73. EXTRACTION AND CHARACTERIZATION OF COLLAGEN FROM SKIN AND BONES OF COD (*GADUS MORHUA*)

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Abstract

Collagen is the main component in fish skin. In fish bones collagen is the major protein. The skins and bones can be collected separately from other by-products. Collagen from mammals in its purified form has found a number of pharmaceutical and cosmetical applications. The quality and specific application of the extracted collagen is highly related to the functional properties and its purity. Known problems with the extraction of collagen from fish skins are the presence of pigments and fish odours, which restrict its potential use.

Here, we will present isolation methods for collagen obtained from skin and bones of cod and the properties of the collagen preparations.

A fermentor was setup for the extraction of collagen below 10 °C. For skins, extraction methods using HCl and acetic acid were compared. The extraction with HCl was more efficient and resulted in a higher yield of collagen. To remove the non-collageneous proteins, pigments and odours the skins were extracted with NaOH and butyl alcohol. The freeze-dried preparation was colourless and pigments were absent.

Cod bones were treated with NaOH to remove non-collageneous proteins. Subsequently, the bones were freeze-dried and ground to facilitate the extraction of collagen. EDTA was added to bind calcium. The concentration of EDTA and extraction time were optimized. NaOH, butyl alcohol and HCl were used similarly as for the skins.

The physico-chemical characteristics of the isolated collagen are described.

Introduction

The demand of collagen and gelatine from the industry throughout the world is considerable and still rising. By-products from fish processing are a potential source of collagen.

Collagen is the main component in the skin (Sikorski and Borderias, 1994), which can be collected separately from other by-products. The major collagen type in fish skin and bones is type I collagen (Sikorski and Borderias, 1994).

Mammalian collagen in its purified form has found a number of pharmaceutical and cosmetical applications. Similarly, gelatine, the hydrolysed form of collagen, is an ingredient extensively used in the food industry. Gelatine is used as a food additive to improve the texture, the water-holding capacity and stability of several food products. Both gelatine and collagen have been derived from fish skins and bones, but have been much less studied than mammalian gelatine and collagen. The quality and specific application of the extracted collagen and or gelatine is highly related to their functional properties and its purity. Known problems with the extraction of collagen from fish skins are the abundance of pigments and the presence of fish odours, which would restrict its potential use. The uniqueness of fish collagen from cold water fish lies in the lower content of amino acids, proline, and hydroxyproline. Although fish gelatine does not form particularly strong gels, it is well suited for certain industrial applications, as for example micro-encapsulations, light-sensitive coatings and, low-set-time glues.

Therefore, the objectives of this study were to develop efficient extraction methods to obtain collagen from cod skins and bones and to characterise the preparations.

Materials and Methods

By-products from processing of cod were purchased from a Dutch fish processor. Preparation of collagen from skin and bones. The preparative procedures were performed below 9 °C.

Skins

The skins were extracted with 0.1 N NaOH at 9 °C to remove non-collagenous proteins, washed with distilled water and centrifuged.

Then, the insoluble material was extracted with 10% butyl alcohol for one hour to remove fat, washed with distilled water and centrifuged. Subsequently, the insoluble material was mixed with water (1:7 w/v) and HCL was added continously to a final pH of 4.0. The soluble fraction was stored at 4 °C for further analysis.

Bones

The bones were extracted with with 0.1 N NaOH at 9 °C to remove non-collagenous proteins, washed with distilled water and centrifuged.

The bones were decalcified with 0.5 M ethylene-diaminetetraacetic acid (EDTA) (pH 7.4) for four hours and centrifuged. The pellet was washed with water and fat was extracted with 10 % butylalcohol for one hour. The insoluble material was mixed with water and HCL was added according to the procedure described for skin.

Rheological measurements

Rheological measurements were carried out using a dynamic stress rheometer SR200 (Rheometric Scientific Inc. Piscataway, USA). Before the measurements the measuring cell of the rheometer was cooled until 8 °C before applying the samples. Rheology was measured in a chromium plate 40-mm parallel plate cell using a gap of 0.100 mm. A dynamic temperature ramp test was performed at a stress of 0.2 Pa and a frequency of 1 Hz. Starting at around 8 °C the temperature was increased with a programmed rate of 2 °C/min. The G' and G" were measured at intervals of 5 seconds. A vapour trap was used to minimise evaporation of water from the sample.

SDS-polyacrylamide gelelectrophoresis (SDS-PAGE)

The collagen samples from bones and skin were dissolved in SDS-sample buffer and electrophoresis was preformed according to protocols of the manufacturer (Biorad).

Results and Discussion

The effect of acid-extraction of collagen has been described for several fish species. Mostly, weak organic acid acids were used. If the pH of the organic acid solution is the most important factor favouring the solubilization of collagen, also other acids, like HCL, could be used. For the practical use in an applied method HCL is cheaper and easier to remove from the collagen extract. Experiments showed that the pH is an important factor in solubilization of the collagen from cod skins and bones. Here, we developed an extraction method for collagen, using a fermentor, with an automatic pH controller.

The HCL soluble fractions were analyzed on SDS-polyacrylamide gels to identify the collagen types. The patterns were similar to type I collagen from Bovine Achillus Tendus. Two bands corresponding to $\alpha 1$ and $\alpha 2$ components were observed. These results are consistent with the collagen type found in the bones and skin of other fish species.

The viscoelastic properties of the collagen in the acid soluble fractions were studied using a rheometer viscometer. The modulus of elasticity (G') and the modulus of viscosity (G") were determined during heating. Changes were observed around 18 °C.

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P 58. NOVEL APPLICATIONS OF COD MILT CATIONIC PROTEINS

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Abstract

In an effort to increase the utilization of fish milt (soft roe), focus was set on the less explored fraction of cationic protein in Atlantic cod (*Gadus morhua*) milt. Today, some milt is used in the production of nucleotides and phospholipids. Novel applications of cod milt cationic proteins could stimulate the interest of exploiting larger volumes of this by-product.

The cationic histone proteins were extracted and partly characterized. In an *in vitro* assay it was shown that Atlantic salmon macrophages, which are known to play an important part in the innate immune system, were stimulated by a crude preparation of histones. A similar effect was found in macrophages harvested from Atlantic salmon that had been injected intraperitoneally with the protein preparation. Further investigation of *in vivo* stimulation was performed by running a challenge experiment with cod fry. Fish given a standard commercial feed supplemented with 0.1% histone proteins were infected by *Vibrio anguillarum.* A significant increase in survival was obtained with fish given the supplemented feed compared to fish given the control feed. The histones also inhibited growth of *V. anguillarum* in a broth dilution assay.

These findings indicate that histones from cod milt can be an attractive additive in feed to help improve survival of fish fry during critical phases.

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P 59. ALASKA WALLEYE POLLOCK BYPRODUCTS: LIPID CONTENT AND COMPOSITION

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Abstract

The total Alaska seafood harvest is over two million MT per year, which results in over one million MT of fish processing byproducts. The fishery for Alaska Pollock (*Theragra chalcogramma*) generates over 700,000 MT of fish processing byproducts per year. These byproducts come from human food processing lines and can be maintained as separate components. Three sets of samples were obtained from commercial plants in Alaska that were filleting Pollock. Samples included whole fish, heads, frames, viscera, and skins. Viscera contained roe and milt. Samples were ground and stored at -70°C until extracted with solvent. An Iatroscan MK-6s was used for percent triglycerides, free fatty acids, diglycerides, monoglycerides, sterols and phospholipids in the oils. Fatty acid profile was determined by GC/FID analysis of fatty acid methyl esters. Data was statistically analyzed and differences ($p>0.05$) reported. Fat content of whole fish was 2.9% and viscera contained the highest levels at 14.3%. Heads, frames and skin presented less than 1.2% fat. Percent phospholipids were lowest for viscera (2.7%) and highest for frames (25.5%). Content of sterols ranged from 9.9% from frames to 15.2% for heads. Triglycerides content was highest in viscera at over 50 %, and monoglycerides ranged for 6.0 to 8% in all samples analyzed. High level of FFA in samples indicated possible lipase activity during storage. The quantity of 24 fatty acids was determined including the long chain omega-3 fatty acids, which ranged from 25.4 to 36.7% of the total fatty acids in all samples. Differences were found in the lipid composition of Pollock byproducts. Composition and lipid content of the individual byproducts is of importance as different byproducts are being used for distinct end products.

Materials and methods

Lipid Extraction, Fatty Acid Methyl Ester (FAME) Analysis and Gas Chromatography.

Lipid extraction was carried out in triplicate as described by Radin (1981), preparation of fatty acid methyl esters was carried out by the method of Maxwell and Marmer (1983), and gas chromatographic identification of FAME was performed on a DB-225 (Agilent Technologies, Wilmington, DE), using a GC 6850 equipped with a flame ionization detector (Agilent Technologies, Wilmington, DE). Three FAME standard mixtures were used to verify retention times, Supelco 37, Marine Oil #1, and Marine Oil #3 (Supelco, Inc., Bellefonte, PA).

Lipid Class Analyses.

Six standards, purchased from Sigma (St. Louis, MO.), were used to identify the lipid classes found in pink salmon oil: cholesterol (ST), triolein (TG), oleic acid (FFA), phosphatidylcholine (PL), diolein (DG), and monopalmitoylglycerol (MG). A five-point calibration curve was determined for each. Hydrogen flow was 60 ml/min and airflow 1.6 L/min. The solvent system was hexane: ethyl ether: formic acid in the ratio of 80:25:1.2 (Ackman et al., 1990). Solvent tank (Shell-USA, Inc., Fredericksburg, VA) was lined with filter paper and solvent equilibration time was 20 minutes. Chromarods-SIII (Iatron Laboratories, Inc., Tokyo, Japan) were cleaned and conditioned for spotting by origin scanning twice at a speed of 50 seconds, and a blank scanning at a speed of 30 seconds. 10 µl of 1 mg lipid per 1 ml chloroform was spotted on each rod and quadruplicate analysis performed for each oil sample. Spotted rods were suspended in the solvent chamber for 10 minutes, eluted for 30 minutes, oven dried for 3 minutes at 110°C, and then scanned (Whitsett et al., 1986). After use, rods were cleaned overnight in concentrated chromic H_2SO_4, rinsed with distilled water, soaked for 3 hours in 5% ammonium hydroxide, rinsed several times with distilled water followed by a final acetone rinse and oven dried for an hour. Clean rods were stored in a 30% humidity chamber until analysis (Parish, 1987).

Statistical Analysis.

The weighted means are derived from an analysis of variance run on Statistica version 6.0 (StatSoft, Inc.). For tests of statistical significance, the data was subjected to Duncan's post-hoc test with significance set at $p<0.05$. Means labeled with distinct capital letters are statistically significant.

Results and Discussion

As shown in the Table1 below, pollock viscera had the highest fat content (14.3%), which was significantly different ($p<0.05$) from whole fish and other tissues, which had fat contents ranging from 2.9 to 0.6% lipids. Percent triglycerides content was also significantly higher in viscera than all other tissues. Percent free fatty acid

values were highest in heads and frames; however, most values were not significantly different. As a generality higher percent free fatty acid values were found in tissues with lower percent total lipids. Percent sterols were not different ($p>0.05$) and ranged from 6.0 to 15.2%. As expected percent phospholipids was lowest in viscera which had the highest percentage of both total lipids and triglycerides.

As shown in Table 2 below, percent saturated fatty acids ranged from 21.4% for viscera to 24.4% for heads. Fillet had the lowest percent of monounsaturated fatty acids (25.1%) and the highest amount of polyunsaturated fatty acids (46.1%), both of which were different from all other tissues ($p<0.05$). The saturated to polyunsaturated ratios (S/P) were 0.9, 0.78, 0.6, 0.85, 0.63, and 0.62 for whole pollock, viscera, fillet, heads, frames, and skin, respectively. As a percent of total fatty acids the percent omega-3 fatty acids ranged from 25.4% for viscera to 45.1% for fillet; values for all tissues were similar ($p>0.05$) except fillet, which was different from other tissues ($p<0.05$). Percent omega-6 fatty acids were low and ranged from 1.7% in heads to 3.1% in skin. The percent of EPA (eicosapentaenoic acid) as a percent of fatty acids identified ranged from 12.3% (whole Pollock) to 16.9% (skin). The DHA (docosahexaenoic acid) values as a percent of fatty acids ranged from 6.4 % (viscera) to 29.2% (fillet).

Table1. Lipid Class Analyses

Pollock Parts	% Lipids	%TG	%FFA	%DG	%ST	%MG	%PL
Whole Fish	2.87 A	39.30 A	6.28 A	22.58 A	13.46 A	7.83 AB	10.55 A
SD Whole Fish	1.16	9.60	8.07	1.10	0.95	0.19	3.66
Viscera	14.32 B	52.39 B	8.17 AB	17.77 A	12.36 A	6.61 AB	2.70 B
SD Viscera	2.86	6.05	0.98	2.91	1.48	1.13	0.79
Fillet	0.60 A	9.36 C	13.79 AB	29.40 B	15.00 A	8.69 A	23.77 C
SD Fillet	0.08	0.71	9.94	5.17	1.24	0.64	3.45
Head	1.15 A	23.30 D	18.05 AB	21.99 A	15.22 A	7.04 AB	14.40 A
SD Head	0.49	9.12	6.50	2.54	5.65	0.74	2.13
Frames	0.96 A	11.50 CD	19.85 B	27.27 BA	9.90 A	5.96 B	25.51 C
SD Frames	0.28	5.88	5.35	4.03	1.41	0.80	7.01
Skin	0.61 A	22.61 D	12.61 AB	31.37 B	14.05 A	8.01 AB	11.34 A
SD Skin	0.16	3.26	3.67	0.66	0.62	0.55	5.05

TG triglycerides, FFA free fatty acids, DG diglycerides, ST sterols, MG monoglycerides, PL phospholipids, SD standard deviation of the mean

Table 2. Summary Fatty Acid Methyl Ester GC Analysis

Pollock Parts	Saturated	Monounsaturated	Polyunsaturated	S/P	Ω 3	Ω 6	Ω 3/ Ω 6
Whole Fish	22.75 AB	47.06 A	25.20 A	0.90 A	33.15 A	2.45 AB	14.67 A
SD Whole Fish	5.48	4.91	2.02	0.18	12.23	0.59	8.08
Viscera	21.37 A	46.66 A	27.77 AB	0.78 ABC	25.37 A	2.10 A	12.14 A
SD Viscera	0.21	4.69	4.47	0.13	4.13	0.29	1.49
Fillet	27.55 B	25.12 B	46.06 C	0.60 BC	45.14 B	2.49 AB	18.26 B
SD Fillet	0.23	1.99	2.23	0.03	1.97	0.23	2.13
Head	24.37 AB	45.15 A	29.15 AB	0.85 ABC	26.96 A	1.72 A	15.85 A
SD Head	1.12	4.60	5.07	0.14	5.26	0.32	2.59
Frames	22.51AB	33.96 C	35.74 B	0.63 BC	35.33 A	2.54 AB	13.89 A
SD Frames	4.01	1.87	6.52	0.04	6.88	0.06	2.72
Skin	21.63 AB	41.72 AC	34.80 B	0.62 BC	36.71 A	3.11 B	12.35 A
SD Skin	1.68	4.11	2.07	0.01	1.79	0.77	3.49

SD standard deviation of the mean

In conclusion, significant differences were found in the lipid content and composition of Pollock byproducts. Composition and lipid content of the individual byproducts is of importance as different byproducts are being used for distinct end products.

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P 60. NUTRITIONAL PROPERTIES OF PROTEIN FROM ALASKA POLLOCK AND PACIFIC COD PROCESSING BYPRODUCTS

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Abstract

The fisheries for Alaska pollock and Pacific cod annually generate over 700,000 mt and 100,000 mt of processing byproducts (Crapo and Bechtel, 2003). These byproducts are easily maintained as separate components during fish processing which could then be used as raw materials for specialty feed and food ingredients. The objective of this study was to evaluate some of the nutritional properties of pollock and cod byproducts.

Three sets of Alaska pollock *(Theragra chalcogramma*) and Pacific cod (*Gadus macrocephalus)* were obtained on separate days from commercial fish processing plants and included; whole fish, heads, viscera, frames, fillets, and skins which were then prepared, and frozen at -80 C. Samples were analyzed for connective tissue content (AOAC meat procedure), amino acid profiles, pepsin digestibility and calculations of an estimated rat protein efficiency ratio (PRE). Data was analyzed using ANOVA and the Duncan post hoc test and differences reported ($p<0.05$).

The connective tissue content of pollock and cod were determined for whole fish (6.8 % and 5.7 %), heads (9.8 % and 13.2 %), viscera (1.0 % and 5.0 %), frames (7.6 % and10.6 %), fillets (1.6 % and 1.6 %) and skins (45.5 % and 42.6 %), respectively. Between species only viscera connective tissue values were different ($p<0.05$). Percent pepsin digestible protein ranged from 92-99 % for all pollock and cod byproducts when using a standard 0.2 % pepsin solution. Calculated rat PER values derived from amino acid profiles of pollock and cod were determined for whole fish (3.0 and 3.0), heads (2.7 and 2.8), viscera (3.1 and 2.8), frames (2.9 and 2.9), skin (1.7 and 1.7) and fillet (3.1 and 3.1), respectively. Between species only viscera PER values were different ($p<0.05$).

Common pollock and cod byproducts generally had similar chemical and nutritional values. These properties are of importance in developing feed and food ingredients and protein concentrates from pollock and cod processing byproducts.

Methods

Samples of fish, fillets, and fish byproducts were obtained from commercial fish processors during the middle of a harvesting and processing season. Alaskan pollock and Pacific cod samples, including whole fish, heads, viscera, frames, fillet, and skins, were obtained over a one week period in February. There are 3 replicates for each byproduct which are samples collected on separate days. A total number of 36 samples (18 for Pollock and 18 for cod) were collected and analyzed.

Fillets were mechanically removed from the headed and gutted fish that was followed closely by mechanical removal of the skins from the fillets. Samples included whole fish, heads, viscera, frames from which the fillets had been removed, skinless fillets (not trimmed) and fillet skins. The viscera samples for cod had the roe and milt removed and the pollock viscera samples contained the roe and milt. Connective tissue content was calculated by first determining the amount of hydroxyproline in sample using an AOAC method (AOAC, 2000). The amino acid content was determined by digesting samples in 6 N HCl for 24 hr at 110 C. Precolumn derivitization (AccQ. Tag, Waters Corporation) was used and amino acid content was determined with a HPLC system. Cysteic acid content was determined after performic acid oxidation followed by hydrolysis in 6 N HCl. Tryptophan content was not determined. The estimated rat protein efficiency ratio (PER) was calculated using the content of ILeu, Leu, Lys, Met, Phe, Thr, and Val on g amino acid per 100 g total amino acid residue basis and the equation developed by Lee et al. (1978). Pepsin digestibility of samples was completed using the AOAC method (AOAC 2000). Fresh solutions of pepsin were made in 0.075M HCl at 0.2, and 0.002 % w/w. After digestion samples were filtered, washed, dried, and the undigested protein was determined as percent nitrogen times 6.25.

Factorial ANOVA procedure were used with Statistica release 6 software with two factors (fish species and byproduct parts) and dependent variables (pepsin digestibility, calculated rat PER, and percent connective tissue). The Multivariate Tests of significance for species, parts, and species*parts were all significant ($p<0.05$). Post-hoc analysis used the Duncan test and the level of significance used was $p<0.05$.

Results

Highest connective tissue values for cod and pollock byproducts were skins ($p<0.05$) and lowest values were for fillet and viscera byproducts. The high content of connective tissue found in heads and frame samples is due in part to the connective tissue in skin and bone components of these samples. Viscera connective tissue content ranged from 1 % for pollock to 5 % for cod. Connective tissue content as a percent of total protein for pollock and cod skins was 45.5 % and 42.6 %, respectively.

Amino acid content of Alaska pollock and Pacific cod heads, frames, fillet, whole fish, viscera, and skin byproducts were determined. As a generality, byproducts for pollock and cod such as heads or skin had similar amino acid profiles. Amino acid analysis of pollock and cod byproducts indicated that whole fish, frames and heads were similar in many respects but different from the fillets, viscera and skin byproducts. Whole fish and head and frame byproducts all contain substantial amounts of bone as evidenced by ash values between 2.6 and 4.6 % (Bechtel 2003). Heads and frames also contain substantial amounts of skeletal muscle such as the collar and cheek muscle tissue in heads, and the muscle tissue was not removed from frame-backbone and the tail regions. Amino acid analysis of pollock and cod skins had substantially higher levels of glycine and proline than all other byproducts and small amounts of the methionine and cysteine. Amino acid profiles of cod and pollock fillets were similar to reported values (USDA, 2001).

An estimate of protein digestibility was determined using digestion with pepsin. When 0.2 % pepsin was used the digestible protein values ranged from 92.0 to 99.1 %. When a 0.002 % pepsin solution was used, the percent digestible protein remained high (91.9 to 99.1 %). These results indicated the fish byproducts had a very high degree of protein digestibility.

The content of seven essential amino acids, ILeu, Leu, Lys, Met, Phe, Thr and Val, were used to determine the calculated rat PER values as shown in the table below. Estimated rat PER values for pollock (1.67) and cod (1.65) skins were much lower ($p<0.05$) than values for other byproducts within a species. High values (3.14 and 3.13) were obtained for pollock and cod fillet. Frames and heads of both species had estimated PER values ranging from 2.64 to 2.94. The estimated rat PER values calculated for pollock and cod frames (2.90, 2.94) or heads (2.73, 2.78) were similar ($p>0.05$). The PER values for cod (2.95) and pollock (3.03) whole fish were similar ($p>0.05$).

Common Alaska pollock and Pacific cod are broadly classified as low fat white fish and in this study the same byproducts generally had similar connective tissue contents, amino acid profiles, and calculate PER values. There were large significant differences between different byproducts within a species. All protein had protein digestibility values in excess of 90 %. These chemical and nutritional properties are of importance in developing feed and food ingredients and protein concentrates from pollock and cod processing byproducts.

Table 1. Calculated Rat PER

	Whole Fish	Heads	Viscera	Frames	Skin	Fillet
Pollock	3.03^{Aacd}	2.73^{Abc}	3.12^{Aace}	2.90^{Aabc}	1.67^{Ad}	3.14^{Aae}
Cod	2.95^{Aabd}	2.78^{Aab}	2.81^{Bab}	2.94^{Aabd}	1.65^{Ac}	3.13^{Aad}

^{AB} Superscripts with same letter within a column are similar ($p>0.05$).
^{abcde} Superscripts with same letter within a row are similar ($p>0.05$).

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P 61. EDIBLE FILMS FROM FISH COLLAGEN –THE EFFECT OF SPECIES AND FISHING GROUNDS ON THEIR PHYSICAL PROPERTIES

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Abstract

With dwindling fish stocks in European waters, there is an urgent need to investigate useful applications of fish waste as potential food ingredients. Edible films have the potential to act as novel biopackaging material as well as effective barriers against moisture and gas in food systems. Collagen and gelatin have many uses throughout the food and pharmaceutical industries and alternatives to mammalian sources are constantly sought. The objective of this study was the investigate the mechanical and water vapour permeability properties of films formed from soluble collagen extracted from cold water species including New Zealand hoki (Macrorunus novaezelandiae), ling (Genypterus blacodes) and Irish cod (Gadus morhua) and ling (Molva molva). Acid soluble collagen was extracted following hand cleaning of the skins to remove any adhering tissue and further purification was achieved by salt precipitation (1.0 M). The solution was centrifuged and the collagen pellet was redissolved, dialysed and freeze-dried. Edible films were formed from plasticised (glycerol:plasticiser = 0.2) or unplasticised protein solutions (1% w/w) of the extracted collagen, poured (35 g) onto level perspex plates and dried under controlled conditions of humidity and temperature (15 ± 1 °C, 50 ± 2 % RH). It was found that films containing collagen derived from NZ species were more permeable to water vapour, more extensible, and had greater tensile strength and elasticity than those formed from the Irish species tested. The addition of plasticiser to the film-forming matrix was found to increase water vapour permeability, extensibility and decrease elasticity in all films but had limiting effects on tensile strength. The differences in physical properties of films between NZ derived collagen and Irish collagen may be attributed to the different fishing climates and the adaptation of the fish to survive in each specific climate

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P 62. ACTIVITY OF A RANGE OF NATURALLY OCCURRING ANTI-OXIDANTS IN FISH OIL SYSTEMS EXTRACTED FROM THE LIVERS OF COD WHITE POLLACK.

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Abstract

Fish oils have been reported to be high in polyunsaturated oils. The use of synthetic antioxidants has been shown to delay oxidative destruction of oil containing systems, but little is know of the effectiveness of naturally occurring antioxidants in fish oil systems. The antioxidant potential of 8 different naturally occurring antioxidants were tested as an alternative to synthetic antioxidants (BHT) on the oxidative stability of oil extracted from the livers of white pollack and cod by cold extraction. The extracted oil was divided into ten 150 ml batches and mixed with eight separate natural antioxidants (0.5 % w/w), a synthetic antioxidant, BHT (0.05 % w/w) and a control. All samples were stored at 30°C in darkness for 16 days. Peroxide, anisidine (a measure of primary and secondary oxidation products in oil, respectively) and totox (peroxide and anisidine combined) values were measured every second day over a 16 day period. Overall. oil samples containing tea catechins or rosemary had significantly ($P<0.001$) lower totox values than oil samples containing the other natural antioxidants. Addition of synthetic and natural vitamin E to the oil system at the selected concentration had a negative effect on the oxidative stability of pollack liver oil. Black clove oil, white clove oil, mustard and carvacol had no significant effect on the oxidative stability of oil extracted from the livers of white pollack or cod. Peroxide, anisidine and totox values were then measured on fresh liver oils samples containing 0.1, 0.25 or 0.5% w/w of tea catechin or rosemary to determine the most effective concentration of anti-oxidant to add to fish oil systems. This research is part of a larger EU funded project QLK1-CT-2000-01017 “Utilisation and stabilisaton of by-products from cod species”.

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P 63. WEIGHT FRACTIONS OF BY-PRODUCTS FROM GADIODS CAUGHT AROUND ICELAND

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Abstract

Five species of the Gadidae family (cod, haddock, saithe, tusk and ling) were caught around Iceland, in April, June and November 2001. Three size categories (length 50-60cm, 60-70cm and 70-80cm) of cod were collected from the southeast of Iceland each time. Additionally, farmed cod and cod (60-70cm) from the west of Iceland were collected in November. The parameters evaluated were physical condition (age, condition factor, length and weight of ungutted fish) and utilization of the fish. The weight of different by-products, like liver, viscera, fillets, heads and more, was recorded and the fractions of theses products evaluated as the ratio of gutted fish.

The ratio gutted/ungutted fish and filleting yield were high for ling and tusk. All size categories of the cod caught in April and the cod from the west of Iceland were high in the ratio of head and viscera. Comparison of the cod caught in November showed that the length and weight of the ungutted fish increased with age of the fish. The condition factor of the wild cod in November was similar for all groups, regardless of size or fishing ground. However the farmed cod had higher condition factor than the wild cod. The cod caught west of Iceland seemed to have a slower growth rate that the cod caught southeast of Iceland, i.e. it was relatively longer and weighted less with regard to age. The filleting yield was also lower.

Introduction

In the fish industry, different products are produced, fillets and so called by-products, including heads, cut-offs, backbone, skin, viscera, liver, roe, and milt. The ratio and characteristics of those products may vary, with specie, fishing ground, season and age of fish. One of the factors used to evaluate the condition of the fish after catch, is the condition factor, which is a certain ratio of the weight and length of the fish after gutting. Processing parameters like filleting yield have been linked to the condition factor of the fish.

Materials and Methods

Five species of the Gadidae family (cod (C), haddock (H), saithe (S), tusk (T) and ling (L)) were caught around Iceland, in April (A), June (J) and November (N), 2001. Three size categories (length 50-60cm, 60-70cm and 70-80cm) of cod were collected from the fishing grounds southeast of Iceland each time. One group was collected for each specie and/or size catagorie for analyses of pooled samples (n=15), except that two groups were collected of the 60-70 cm fish (Cod 60), one for analyses of pooled samples, the other for analyses of individuals (CodI). Additionally, farmed cod (NC-F) and cod (60-70cm) from the fishing grounds west of Iceland (NC-W) were collected in November and analysed individually. The parameters evaluated were physical condition and utilization of the fish (n=15), i.e. age (only of the fish caught in November), length, c-factor, condition, weight of ungutted fish, ratio of gutted fish compared to ungutted fish, and ratio of by-products with regard to gutted fish.

Results and Discussion

Variation in filleting yield and the ratio of head and viscera caused the greatest difference between the species. Ling was highest in filleting yield whereas the cod caught in April had the lowest yield. Filleting yield was negatively correlated to ratio of head and viscera. Comparison of the cod caught on fishing grounds south east of Iceland showed the filleting yield was higher in November than in other months.

The condition factor in November was similar for the wild cod regardless of size or fishing ground. However, the farmed cod had significantly ($p<0,05$) higher condition factor than the wild cod. The length and weight of the ungutted fish in November, increased with age of the fish. The cod caught west of Iceland seemed to have a slower growth rate than the cod caught southeast of Iceland, i.e. it was relatively longer and weighted less with regard to age. The filleting yield was significantly lower than of fish caught on fishing grounds southeast of Iceland and farmed cod.

Two different methods were used for the calculation of condition factors for cod, the c-factor and condition. C-factor was evaluated as the ratio of weight compared to length in 3rd power (Fulton, 1903). This factor has been modified with regard to decreased growth rate of fish with age, for evaluation of condition (Brynjólfur Gísli Eyjólfsson, 2001). The results indicated that changes with size, season and fish grounds were, similar for both factors.

Conclusions

The ratio of by-products varied with species and seasons. Seasonal variations were observed in filleting yield and condition factors of cod, which were highest in November and lowest in April. The length and weight of the cod caught in November compared to age varied with fishing grounds. The farmed cod had higher condition factor than the wild cod but the cod from fishing grounds west of Iceland had the lowest filleting yield.

Results from the EU-project: Utilisation and stabilisation of by-products from cod species (QLK-CT2000-01017)
Project manager: Turid Rustad, Department of Biotechnology. The Norwegian University of Science and Technology.
Other partners: SINTEF, Norway, UCC, Ireland, IFREMER, France, RIVO, Netherlands and NOVOZYMES AS, Denmark.

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P 64. UTILISATION AND STABILISATION OF BY-PRODUCTS FROM COD SPECIES

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Abstract

In this project, by-products from five cod species (cod, saithe, haddock, tusk and ling) is being studied. The work is focused on the protein and lipid fractions from viscera, liver, skin, bones and cut-offs and on methods to sort, handle, extract valuable parts and to stabilise the fractions.

The main objectives are:

1) Develop value added food ingredients from by-products from cod species
2) Develop systems to recognise and separate different fractions
3) Characterise the chemical composition of different by-products
4) Develop suitable methods for preserving the different fractions
5) Develop methods to extract the interesting fractions/biomolecules.
6) Where appropriate to develop the capacity to do all of the above on board vessels to optimise quantity and quality of high value added by-products
7) Review the market potential for the selected by-products

The by-products has been collected from different parts of Europe and transported frozen (<-30 ^{o}C) to the partner/laboratoratories for chemical analysis and experiments. Weights of different by-product fractions have been determined. These data is being utilised in a computer program calculating the possible production of fish rest raw materials onboard fishing boats with different quotas. Characterisation of the lipid composition and protein composition of the by-products have been carried out. Sorting of by-products using robotic technology is being developed and looks very promising.

Protocols for extraction of collagen have been developed. A marketing study on the industry needs regarding collagen properties are in progress together with work to increase the heat stability of collagen. Trials to use fish proteins hydrolysates to improve water-holding capacity of fish fillets have been made.

Extraction of valuable parts (lipids and proteins) is being carried out using different methods: enzymatic, chemical and water extraction both in lab and pilot scale. It is possible to partition triglycerides and phospholipids by simple processes. Screening of lipid fractions for bioactive compounds are in progress and some active fractions have been found.

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P 65. DOCUMENTATION AND NOVEL FUNCTIONS OF MARINE BY-PRODUCTS

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Abstract

A relationship between fish consumption and reduced mortality due to cardiovascular diseases has been shown. Curiously, in most studies the positive effect has been attributed to the intake of marine fatty acids alone, although lean and fatty fish have given the same protective effects in many studies. The presence and significance of additional beneficial components from seafood have attracted attention lately. Components like marine derived tocopherols and quinones, selenium, magnesium, ACE-inhibitors, taurine and marine peptides and proteins have attracted special attention due to their contribution in prevention of low-density lipoprotein (LDL) oxidation.

Processing (including preparation) makes food healthier, safer, tastier and more shelf-stable. While the benefits are numerous, processing can also be detrimental, affecting the nutritional quality of foods [1]. Removal of molecules that causes off- flavours or taste to improve sensory attributes may, for instance, destroy potent antioxidants in muscle foods. Modern meal preparing techniques may also lower the content of biologically active components. Blanching, for example, results in leaching losses of molecules (vitamins, minerals, amino acids, peptides i.e.). Heat treatment is one of the most important factors influencing food quality and the availability of nutrients in the food. The result can be measured by a decrease in both the digestibility and biological value of the food [2-6]. Oxidative reactions are enhanced. Many of the components suggested as additionally beneficial nutrients from seafood are affected by thermal processing; free amino acids, peptides and proteins are all affected by thermal processing. The processing technique will also affect the availability of nutrients in the food. The nutritional quality of the food depends on quantity as well as availability of such molecules. The time and temperature of processing, product composition and storage are all factors that substantially impact the status of our foods.

Objectives

The principal objective is to study the possibilities of making a minimal processed marine product with elevated levels of beneficial components. The project will document the impact of different low temperature drying and separation techniques on nutritional and functional properties. Process combinations that optimise safety, shelf life and nutritional and sensory properties will be selected.

Methods

Fishery by-products from cod and salmon will undergo drying techniques such as spray-, flash- freeze- and vacuum drying.
Various filtering techniques such as micro-, ultra- and nanofiltration plus reverse osmosis will be performed on liquid fractions.

Analyses of the biological material before and after processing will be performed on a selection of low molecular components such as taurine and ACE-inhibitors by use of HPLC.

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P 66. PROTEIN ISOLATE FROM EXOSKELETON MATRIX OF BRAZILIAN PINK SHRIMP (*FARFANTEPENAEUS PAULENSIS*): EXTRACTION AND CHARACTERIZATION

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Abstract

Wastes of crustacean industrialization in the port of Rio Grande, south extreme of Brazil, had become an increasing problem due to deposition of them, near to Patos Lagoon or industrial installations, therefore its processing liberates up to 85% of residues when mechanically peeled. Protein included in the matrix of shrimp exoskeleton, associated with the astaxanthin, chitin and calcium carbonate, has not been very studied at Brazil, being its presence of interest, because it could arrive up to 40% of the organic substance of the shell. This work had as objective, to study the parameters related with a process of chemical extraction and characterization of protein isolate from Brazilian pink shrimp exoskeleton matrix. Previous processing of the raw material included its selection, washing, drying and milling. In the extraction phase, protein was recovered by immersion and agitation in alkaline means and acid precipitation after to reach the isoelectric point (at pH 4.5). To define this stage, a factorial planning was executed where better parameters had been: extraction-time 20 minutes, extraction-temperature 40^o C, in 2 stages, concentration of alkaline agent 0,25%. After, the product was dry in forced-air circulation oven, triturated and reserved for analysis. The final isolate presented 89.02 % of protein (dry basis), 33.20% of solubility in alkaline pH and digestibility coefficient "in vitro" of 98,37%. The essential amino acids in higher concentration were leucine (6,15%), valine (5,14%) and lysine (4,99%), with excellent potential for use in protein formulations, adding value to crustacean by-products.

Introduction

Wastes proceeding from the shrimp industrialization present components that could be usable, amongst them, chitin, astaxanthin and protein (Meyers, 1987, Prentice and others, 1999). Protein associated with the chitin and calcium carbonate, present in the shrimp exoskeleton matrix, has not been widely studied, but some authors denounce its presence with interest, because it could reached up to 40% of the shell organic matter (Cano-Lopez and others, 1987; Martin, 1994; Meyers 1986; Tenuta F^o and Zucas, 1987). They estimated that it can be recovered of the wastes for alkaline extraction and acid precipitation when reaching the protein isoelectric point, and after using it in the complementation of food formulation for human consumption (Meyers, 1986; Tenuta F^o and Zucas, 1987).

Nutritional value of the protein present in the shrimp waste is considered high, presenting similar amino acids profile to the casein, for that reason, the recovery process can be considered attractive (Martin, 1994).

Material and Methods

Shrimp wastes coming from it mechanical peeling was conditioned and carried to the FURG Laboratory of Food Technology, where they was submitted to washing, selection, physical and chemical characterization was realized. After characterization, waste had been dried in forced air circulation oven to 45ºC for 12 h, then grinded and the adequate particle size determined. Extraction process of protein consisted of alkaline treatment, where the control parameters was: particle size, alkaline agent concentration (0.25, 0.5, 0.75 and 1%), process time (30, 60, 90, 120 and 180 min), temperature (30, 45 and 60ºC). After extraction period, a centrifugation at 6000 rpm for 15 min, was realized, the solid residue was submitted to 2 new extractions in the same conditions explained previously and the cake discarded. The liquid phase was submitted to the acid precipitation after to reach the isoelectric point of the protein (between 4.5 and 5.0), where the control parameters were: precipitant agent and its concentration (0.5, 0.75 and 1 M), time (10, 20, 30 and 40 min), ionic force (addition of precipitant agent in concentration of 0.05, 0.5 and 0.1 M), initial and final temperature (30, 40 and 50ºC) and pH (6.0, 5.5, 5.0, 4.5 and 4.0). New centrifugation was realized, where the superior phase was discarded and the precipitated product was submitted to the drying in oven at 45^o C for 12 h, then, it was realized the physical (pH) and chemical (protein, humidity, lipids, ash and fiber) values for posterior determination of digestibility coefficient allowed to determine the nutritional and functional properties (solubility and water absorption and retention), respectively.

Characterization according to Brazil (1981) was realized, raw fibers (Brazil, 1991), TBA number and peroxide index according to AOAC (1995), solubility determination following the method of Morr and others (1985), *in vitro* digestibility coefficient and water absorption and retention by method indicated for Regenstein and others (1984).

Results and Discussion

Pink shrimp waste utilized as raw material had presented the pH value 7.66, TBA number (mg MA/Kg of lipid) 0.06 and peroxide index (meq. peroxide/Kg of lipid) as 8,84, demonstrating to be apt to continue the process steps (Prentice and others, 1999; Costa and Prentice, 2002). The proximal composition is shown in Table 1.

Table 1. Proximal composition of pink shrimp exoskeleton as raw material

Constituents	% WB	% DB
Moisture	79,98	-
Protein (N x 6,25)	8,84	44,15
Lipids	0.17	0,87
Ash	4,45	22,22
Fibers	6,56	32,76
Total	100,00	100,00

WB = Wet basis; DB = Dry basis

Process yield was 69 %. Recovered protein presented a digestibility coefficient of 98.37%, water absorption index (291.78 WAI) and water solubility index (WSI) 2.73, showing its potentiality in the use in protein food formulations (Costa and Prentice, 2002; Costa, 2003). Table 2 presents the proximal composition of the recovered protein.

Table 2. Proximal composition of shrimp protein isolate as final product

Constituents	% WB	% DB
Moisture	4.71	-
Protein (N x 6.25)	84.83	89.02
Lipid	9.36	9.84
Ash	366	3.85
Fibers	6.50	6.83
Total	100.00	100.00

WB = Wet basis; D B = Dry basis

The protein isolate, obtained as final product, was a reddish powder, odorless, and very hygroscope power.

Conclusions

The developed process from protein isolate extraction of shrimp wastes presented as optimal parameters: extraction temperature, 45ºC; extraction time, 180 min, alkaline agent concentration, 0.25%; isoelectric point pH, 4.5 using HCl as acidifying agent at 40ºC for 20 min.

The protein isolate obtained presented 89.02% of protein in dry basis, digestibility coefficient 98.37%, foaming stability 75.73, foaming capacity 237.1, water retention capacity 1.1 at pH 2 and 50^{o}C. Essential amino acids in higher concentration were leucine (6,15%), valine (5,14%) and lysine (4,99%)indicating high potentiality in the use of food formulations for human consumption.

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P 67. Volatile compounds in artificial bait based on fish by-products

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Abstract

Volatile compounds of several types of bait were characterized to study the release of attractants that contribute to fresh fish odor. The aim is to develop bait composed of raw material that will have similar or more release of attractants than traditional bait. The objective was to develop bait from raw material used for meal production and byproducts from fish processing, for the long-lining deep-sea fishing fleet, mainly cod, haddock and hake. Volatile compounds were measured by using gas chromatography (GC/Olfactometry and GC/Mass Spectrometry). Fatty acid, free amino acid and chemical composition of bait were also measured. Variation in the key aroma compounds identified in bait explained the difference between bait samples and similar differences could be explained by the fatty acid, free amino acid and chemical composition.

Introduction

The development of successful bait for marine commercial species is an aim for many researchers dealing with chemoreception. Regarding gadiformes (cod, hake etc.) it is believed that its feeding behaviour is primarily mediated by chemosensory mechanisms (Pawson 1977). Although different types of substances are known to provoke responses in fish, chemosensory research involving behavioural and electro-physiological work have given strong support that free amino acids (specially L-stereoisomers) and other low molecular weight components of tissues are dominant in the aquatic environment in this respect (Ellingsen and Doving 1986).

Gadiformes fish live on live prey and are therefore very sensitive towards freshness. Preparation of bait material, like thawing, grinding mixing etc. seems to destroy the chemoreception that attracts the fish to the bait. It is known that enzyme activity (lipoxygenase and protease) in fish is involved in producing degradation compounds (flavor compounds and amino acids) (Josephson and others 1987) that may attract the fish when used in bait. The role of volatile compounds as attractants in bait has not been studied before.

Materials and Methods

Samples. Volatile compounds of different types of traditional and artificial bait were analyzed using gas chromatography/olfactometry (GC/O) and gas chromatography/mass spectrometry (GC/MS). Fatty acid, free amino acid and proximate analysis were done using traditional methods.

Sampling and gas chromatography techniques. Prior to GC/O and GC/MS analysis flavor samples were collected by a purge-and-trap sampling on Tenax traps (Olafsdottir and others 1985). Volatile compounds were separated on a fused silica capillary column, DB-5ms (J&W Scientific, Folsom, CA) using helium as a carrier gas. Details of the technique and identification of the volatiles are described elsewere in the proceedings (Jónsdóttir and others 2003).

Data handling. Multivariate analysis was performed by the Unscrambler 7.5 software package (CAMO AS, Trondheim, Norway). The main variance in the data set was studied using Principal component analysis (PCA). Cross validation was used in the validation method

Results and Discussion

Figure 1 shows a PCA biplot of the main volatile compounds identified in bait by GC/O. The figure shows that the combination of volatiles were similar in cybrine and cybrine waste, indicating that the cybrine waste could be used as a bait instead of cybrine. Cod viscera and blue whiting are most dissimilar from cybrine. The capelin samples and the sandeel are positioned near the middle on the first PC indicating their similarity. The squid sample had volatile composition unlike the other samples and had rather mild odor.

Boiled potato-like odor (7-potato) was identified as the most important odor in all the samples, except for squid where this odor was not detected. Other important odors were i.e. popcorn-like (8-popcorn), mushroom-like (10-mushroom) and viscera-like (15-viscera) odor. Volatiles that are characteristic for all the samples, i.e. mushroom, are in the middle of the PCA plot and do not contribute to the variation in the data.

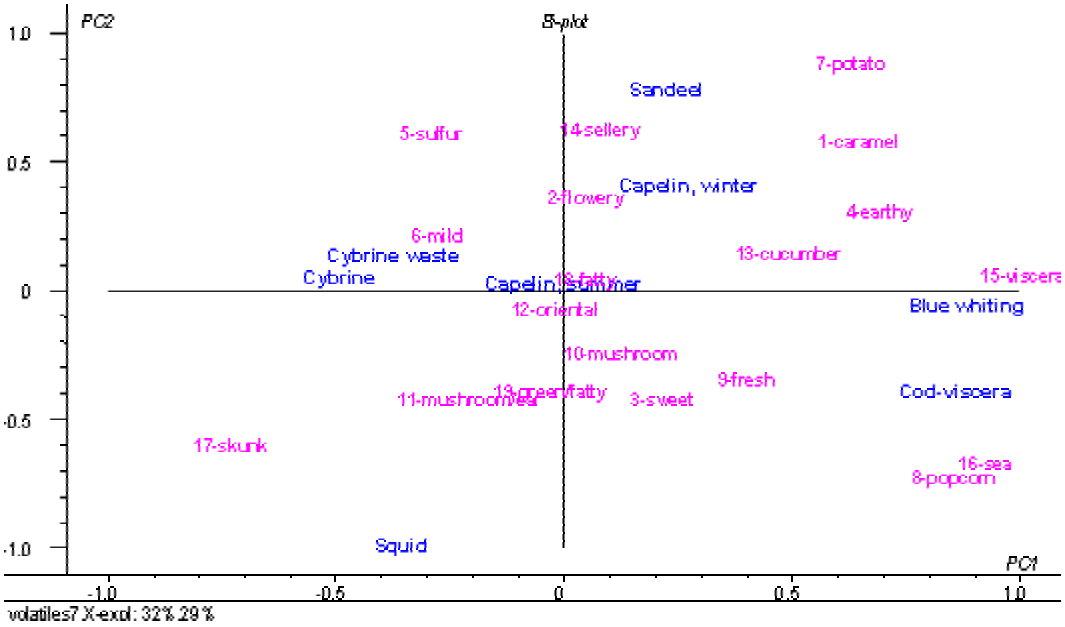

Figure 1. PCA biplot of volatile components in traditional and artificial bait by GC/O. The numbers in odor descriptors give the compound no in table 1.

Table 1 shows the most characteristic volatile compounds identified in bait by GC/O and GC/MS. The boiled potato-like odor was identified as a combination of *cis*-4-heptenal and heptanal eluting close to each other from the column. Lipid derived components giving mushroom and cucumber odors were identified as 1-octen-3-ol and 2,6-nonadienal, respectively. Many compounds are still unidentified and need to be studied further. The results of fatty acid, free amino acid and proximate analysis were in agreement with the volatile composition and could explain the difference between the species.

Table 1. The most characteristic volatile compounds identified in bait by GC/MS and GC/O.

Compound no.	Possible compound	RI DB-5ms[a]	Odor	Identifiaction[b]
7	cis-4-heptenal and heptanal	496	Potato, mushroom, geranium, shellfish, flesh	MS, 1, 2
8	2-acetyl-1-pyrroline	521	Pop corn	3
10	1-octen-3-ol	581	Mushroom, geranium, capelin	MS, 1, 2
12	ui	687	Oriental, spicy	2
13	2,6-nonadienal	760	Cucumber	MS, 1, 2
14	ui	776	Sellery, mild sweet	2
15	ui	797	Viscera, cybrine, heavy, vomit, waxy	2
16	ui	800	Sea-, shellfish-like, heavy	2
17	ui	826	Skunk	2

[a]Calculated ethyl ester retention index on DB-5ms capillary column

[b]Identification means: MS, mass spectra; 1, authentic standards; 2, odor identification; 3, odor identification and RI references

ui: unidentified

Conclusion

Variation in the key aroma compounds identified in bait explained the difference between bait samples and similar differences could be explained by the fatty acid, free amino acid and chemical composition.

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K 9. INFLUENCE OF FEED AND FEEDING PRACTICE ON QUALITY FACTORS SUCH AS NUTRIENT COMPOSITION AND EATING QUALITY OF AQUACULTURE SPECIES

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Introduction

Nutritional quality of food is related to the content in nutrients, their bioavailability and their ability to meet the human requirements. Fish flesh contains highly digestible protein (17–22 g/100 g wet weight) with a well balanced amino acid profile, is rich in free amino acids (1.3 to 3.8 g/100 g) and low in collagen (3 to 10 g/100 g compared to 16 to 28 g/100 g in beef meat) and supplies a variety of minerals and vitamins (Médale et al., 2003). However, due to the increasing evidence of the healthy benefits associated to n-3 fatty acids consumption, the main interest of eating fish lies in its content in typical n-3 long chain poly unsaturated fatty acids (PUFA). While protein level and amino acid profile of fish are nearly constant, micronutrients content, lipid level and fatty acid composition are greatly affected by feed composition and feeding practice.

Eating quality is an important determinant of food choice. If the product does not look attractive, does not smell good or does not taste good, the consumer does not purchase it despite its nutritional and healthy value. Sensory quality is more complex to define than nutritional quality because it is not directly related to one single flesh component. It results from a set of sensory characteristics such as color, texture, odor and flavor which can be evaluated by instrumental measurements or by eating perception. The desired flesh characteristics for particular species varies between markets and may differ significantly for raw and processed products (cooked, smoked or salted products). Fish nutrition, by affecting the composition of fish body, has a great impact on the overall quality criteria. This paper summarizes the impact of some major feed components on flesh quality of fish.

Macronutrients

1 - Protein levels and protein sources

Among the farmed animals fish are one of those that require the highest level of dietary protein for growth and protein cost is the main component of the fish feed price. The effect on body composition of feeding various levels of protein has been examined in several studies. Protein content of whole body and muscle appears to be solely dependant on fish weight and to be fixed within a small range for each species at a given size regardless of the diet composition provided that fish is growing (Reinitz, 1983; Shearer, 1994). High levels of dietary protein result in an increased fat deposition. When protein intake exceeds the level that fish is able to use to synthesize body protein, carbons from dietary protein are partly stored as fat. Dietary protein levels seem not to affect texture of fish muscle. Positive correlations between muscle fibers density and texture have been observed in several species. Muscles with a great number of small fibers would be firmer than muscles with fewer larger fibers (Johnston, 2001). Johnston et al. (2002) did not observe any effect of the dietary protein levels (crude protein : 48 to 44 and 42 to 37 % dry matter) on fiber number and fiber size distribution.

Fish meal with a high protein content and a well balance amino acid profile to meet fish requirements is the basis of most farmed fish feeds. In order to decrease the reliance of aquaculture on the fish meal derived from fisheries, numerous studies have been conducted to partially or totally replace fish meal by other protein sources. Surprisingly, few of these studies were aimed at evaluating the consequences of fish meal replacement on quality attributes of flesh fish. A work conducted on Atlantic salmon fed either fish meal diet or a diet where 13% fish meal was replaced by full-fat soybean meal showed that dietary protein sources did not significantly affect proximate composition, fat deposition, the amount of astaxanthin in the muscle, visual color score or sensory characteristics (Bjerkeng et al., 1997). Organoleptic tests performed by a human taste panel on rainbow trout fed up to 250 g either on a fish meal based diet or plant protein (soybean or cottonseed meal) diets led to the same conclusion (Smith et al., 1988). However, Kaushik et al. (1995) reported some differences in sensory characteristics between groups of rainbow trout fed diet containing either 100 % fish meal or 100 % soy protein concentrate as the protein source. According to these authors, total replacement of fish meal by soy protein concentrate gave a more freshwater taste of the flesh. Off-flavors due to the presence of powerful flavor compounds seems to be due to the total amount of plant products in the diet since Bjerkeng et al. (1997) mentioned that no off-odors or off-flavors were detected by the sensory panel that evaluated salmon fed a diet with 13% soybean meal. Plant protein sources do not have the same amino acid profile as fish meal. It has been demonstrated that the free amino acids pool of fish muscle varies with the dietary amino acid supply. Free amino acids and peptides probably make some contribution to flavor.

2 - Lipid levels and lipid sources

Developments in feed technology have led to the production of feeds of high nutritive value that contain high energy levels. The energy enrichment of fish feed has been achieved through the increase in lipid levels, lipid being more concentrated in energy and more digestible than carbohydrates. There is a general agreement that increasing dietary fat level improves protein retention by reducing protein catabolism but also results in increased fat deposition in fish whole body. However, the site of lipid depots vary among species : lean fish as cod stores fat mainly in the liver (up to 75 %), fatty fish such as herring, mackerel or eel deposit great amounts of fat in the muscle while in intermediate fish like salmonids lipids are stored both in perivisceral adipose tissue and in the muscle. Diet induced changes in lipid storage mainly affect the predominant site of deposition. In salmonids, the relationship between fillet fat content and the dietary lipid level is not clearly defined. Some studies reported that high fat diets increases fillet fat content in Atlantic salmon (Hemre and Sandnes, 1999), rainbow trout (Rasmussen et al., 2000) and brown trout (Regost et al., 2001). Differences reported in the literature could be related to the size of the fish and the capacity of intake of high energy diets. It seems that fillet fat content of large Atlantic salmon reaches a plateau at high dietary lipid levels. According to Einen and Skrede (1998) the threshold would be 34 % dietary fat but Hemre and Sandnes (1999) observed an increased fat deposition in the muscle of Atlantic salmon fed higher lipid levels (up to 47 %).

The effects of high fat diets on sensory characteristics of the flesh is still a matter of debate. Some studies conducted with different salmonid species indicated only minor diet-induced variations in flavor and texture attributes of cooked or smoked fillets despite the fat content of the fillet was changed (Bjerkeng et al., 1997; Rasmussen et al., 2000; Régost et al., 2001). However Robb et al. (2002) demonstrated that texture and flavor attributes were significantly correlated with the fillet fat content of Atlantic salmon. When negative effects appear (oily texture, intense fishy flavor), they can be reduced by feeding the fish with a low-fat diet prior slaughter. Low-fat finishing diet seems to be a feeding practice more efficient than long term starvation at improving the overall quality characteristics of fish (Rasmussen et al., 2000; Rasmussen, 2001).

Numerous studies have demonstrated that the fatty acid profile of the diet has a strong impact on the fatty acid profile of the lipid deposited in muscle. However the variation in flesh fatty acid content is typically less pronounced than that of diet (Bell et al., 2003; Régost et al., 2003a) . This is of particular concern since fish is a unique n-3 PUFA rich food product for human consumption. As aquaculture consumes a large proportion of the fish oil available on the market, alternatives to fish oils in fish diets have received considerable attention in the recent years. The fatty acid composition of vegetable oils differs deeply from that of marine fish oils. Substitution of fish oil by vegetable oils in the fish feed dilute the EPA (C20:5 n-3) and DHA (C22:6 n-3) levels in fish flesh and increases the content in C18:2 n-6 with consequences on taste, healthy values and consumer acceptance.

A promising feeding strategy to reduce the use of fish oil for aquaculture without compromising fatty acid profile of flesh fish is the return to a fish oil diet some weeks prior slaughter (Bell et al., 2003; Régost et al., 2003a). According to an ongoing study performed under an European research project (RAFOA; Corraze et al., personal communication), feeding for 3 month with a fish oil diet rainbow trout previously fed for 9 month a 100 % vegetable oil (rapeseed or linseed oil) diets allows to restore 80 % of DHA and EPA but wash out of C18:n-6 was less efficient. Further research is needed to find the right balance in order to produce fish with the desired fatty acids profile.

It is worth to note that total substitution of fish oil by vegetable oil does not impair growth or fat deposition. There are contradictory reports about the impact of feeding fish with vegetable oils on the sensory attributes of the flesh (Rasmussen, 2001) but when some changes in organoleptic properties are observed, they can be reduced with a return to a fish oil based diet (Régost et al., 2003b).

Micronutrients

Although a number of micronutrients contained in flesh fish can be affected by food supply, this part will be restricted to some of them that are thought to have a major impact on flesh quality.

1 - Vitamin E

Among the lipid soluble vitamins, tocopherols are of major interest because their main function is to protect tissues unsaturated fatty acids against oxidation by blocking the free radicals reaction chain. Oxidation of fish lipids is a major problem of flesh quality : it can decrease the nutritional value of the product by lowering the content in PUFAs, produce off-flavor compounds and modify texture and color. Several studies conducted in different fish species demonstrated that deposition of lipid-soluble vitamin such as vitamin E in the muscle was dependant on the dietary supply (Frigg et al., 1990; Bai and Gatlin, 1993; Sigurgisladottir et al., 1994). Fish muscle enriched in vitamin E by increasing dietary tocopherol level has an improved oxidative stability during storage. Concentration in malonaldehydes that result from lipid oxidation, is maintained low when the flesh content in vitamin E is high (Frigg et al., 1990; Bai and Gatlin, 1993; Baker, 2001). The main source of aroma in fish being the compounds formed by the oxidation of PUFAs, increasing the concentration in vitamin E in the feed leads to reduce the “fishy” aroma of fish fed high fat diets (Chaiyapechara et al., 2003).

2 - Vitamin C

In fish, individual muscle fibers and fibers blocks (myotomes) are surrounded by connective tissue (myocommata) which is mainly composed of a network of collagen sheets connected by tubules of collagen. Ascorbic acid is essential in the formation of connective tissue as a cofactor in the synthesis of hydroxyproline, a

precursor of collagen. Consequently, high vitamin C content was expected to improve muscle integrity thus limiting slits or holes at the surface of the fillet. This phenomenon known as gaping is caused by the rupture of collagen tubules where they join collagen sheets. Although muscle content in vitamin C increases with the dietary content in vitamin C (Merchie et al., 1996; Gouillou-Coustans et al., 1998), the beneficial effect on reducing gaping has not been demonstrated. According to Baker (2001), it is likely that flesh pH, fat and water content predominate on vitamin C as the key factors that govern muscle integrity in addition to slaughter and post-mortem storage conditions.

3 - Carotenoïds

The orange-red color of salmonid flesh is a criterion of great economical importance. In aquaculture carotenoid pigments must be provided by food since fish are not capable of synthesizing these pigments. There are many potential sources of carotenoid pigments : yeast, algae, krill or industry derivatives such as crab and shrimp offals but these sources result in highly variable pigmentation due to the high variability in pigment content and bioavailability. The main carotenoid used in aquaculture is astaxanthin which is sometimes associated to canthaxanthin. However supplementation with these synthetic molecules increases the cost of food by 15 to 30 %.

Pigment fixation in the muscle depends on factors such as concentration of the pigment in the diet, level of dietary lipid, digestibility of pigment sources, duration of the supply and the aptitude of the fish for depositing pigments (Choubert, 2001). Fixation of astaxanthin in the muscle increases with the amount of ingested astaxanthin up to a plateau. The color of trout and salmon muscle then tends towards a maximum that cannot be exceeded. Choubert and Storebakken (1989) found that the maximal retention of astaxanthin was 12.5 % of the ingested dose in rainbow trout fed a dietary level of 25 mg per kg food. In the EU, the maximum recommended level is 100 mg of astaxanthin per kg of feed.

In addition, since carotenoids are lipid-soluble compounds, their absorption is linked to that of lipids. Adding fat to the food favors pigmentation through an improvement of pigment digestibility. The effect of lipid sources on muscle pigmentation is still not clear. According to Choubert (2001), the concentration of astaxanthin in trout muscle would not be affected by the origin of dietary fat (corn oil vs fish oil).

Conclusion

Feed components and feeding practice strongly affect the lipid components of the flesh that give to the product its healthy value and pigmentation that is a major quality criterion of salmonids. Feeding strategies can also modify some other quality parameters such as texture, odor and taste but to a lesser degree than do slaughter techniques, storage conditions and processing. Despite increasing concern in optimizing quality of farmed fish, we still are not able to forecast the characteristics of the products. A major goal of future research is to elucidate the mechanisms that govern the diet-induced changes in flesh components and that determine the different sensory attributes.

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L 74. CHARACTERIZATION OF SQUID HYDROLYSATE FOR ITS POTENTIAL AS AQUACULTURE FEED INGREDIENT

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Abstract

In the Northeast of the U.S., approximately 36,000 MT of squid (*Illex illecebrosus* and *Loligo pealei*) has been landed annually. It is estimated that the processing byproducts (SPB) account for approximately 40% of the raw squid weight. The protein constitutes 72-77% of solid mass of byproducts. At this high protein level, the most viable approach to full utilization of SPB would be a bioconversion into hydrolysate as a nutrient additive. Hydrolysis by its endogenous proteolytic enzymes makes the process more cost effective. In order to establish an optimum hydrolysis condition, changes in the degree of hydrolysis (DH), viscosity, electrophoretic patterns of protein and peptides, and free amino acid profiles of the ground SPB were monitored during hydrolysis at 55°C and natural pH (6.8). The DH value markedly increased from 10.17-10.49 to 16.5-18.7 upon 2 hr hydrolysis, where the initial high DH value reflects the rapid initiation of hydrolysis upon mechanical homogenization prior to the heat-assisted reaction. Viscosity of the hydrolysate exponentially decreased up to 2 hr. The progress of hydrolysis was successfully monitored by viscosity measurement. The disappearance of large molecular proteins > 21.25 kDa coincided with the marked decrease in viscosity and increases in DH and free amino acids. The 2-hr hydrolysis resulted in 2.36-fold increase in the total free amino acid contents. Hydrolysate with protein MW < 45 kDa appeared to have an attractability to trout fingerings. Feeding trials on Atlantic salmon juveniles and sac fries indicated improved survival rate and feed conversion. The SDS-PAGE revealed that 2-hr hydrolysis with a DH value of around 17 yielded peptides as the major fraction with a small fraction of partially hydrolysed proteins which is believed to be a contributing factor to an optimum nutrition for fish growth.

Introduction

The 40% of incoming raw squid ends up as byproduct during a typical cleaning and dressing process in which mantles and tentacles are separated for food use. The byproduct largely consists of head, fin, and viscera along with unclaimed mantles and tentacles. It contains approximately 11% protein, 2% lipid, 1.3% ash and 86% moisture. The level of protein is high enough for proteolytic hydrolysis (enzymatic digestion) to generate large amounts of peptides and free amino acids responsible for feed attraction and growth stimulation. One of unique features of this process is the use of endogenous enzymes for hydrolysis with no commercial enzymes added. Reportedly, inclusion of 5-15% squid meal increased survival and weight gain for many Penaeid species (Joseph and others 1987) owing to squid protein fraction (SPF) that has shown a growth-promoting effect in shrimp at levels from as low as 1.5% which was later related to an unknown "growth factor", possibly low m.w. peptides (Meyers, 1989). There have been little studies on squid as finfish feed ingredient. The objective of our study was to characterize the squid hydrolysate for its potential as aquaculture feed ingredient by examining hydrolysis condition and hydrolysate protein profile as well as conducting fish feeding.

Materials and Methods

Fresh squid processing byproduct was chopped in a Hobart meat cutter, and placed into a stainless steel 40 x 40 cm vessel. The vessel was then put into a 55°C water bath (set up in a laboratory retort unit) and hydrolyzed at natural pH (6.8). Samples were taken at varying intervals for analyses of degree of hydrolysis (OPA reagent, Nielsen and others, 2001), viscosity, the electrophoretic patterns of proteins (SDS-PAGE) (Laemmli, 1970) and peptides (1-100kDa, Hermann and Gebhard 1987), the HPLC-amino acid profile and attracting properties. For attractability on trout fingerlings, the cotton balls soaked with hydrolysate and control (distilled water) enclosed in perforated hollow plastic golf balls were placed in the both sides of aquarium for the release of attractants. After 2 min, the fish appeared were counted in the next 5 min.

Results and Discussion

The progress of hydrolysis was monitored by measuring viscosity changes. Based on the relationship of viscosity changes to hydrolysate protein profile (SDS-PAGE), hydrolysis was terminated when the viscosity reaches ~200 cP, immediately followed by heating to 75°C for 30 min to stop further hydrolysis. The resulting pasteurized hydrolysate had a viscosity of ~ 80 cP. The marked reduction of viscosity was a result of additional hydrolysis that took place in the initial part of termination and heat-induced protein denaturation. At this point, a maximum yield of hydrolysate with desired molecular weight (< 45 kDa) for feed attractability and growth stimulation was achieved. The SDS-PAGE revealed that 2-hr hydrolysis with a degree of hydrolysis (DH) value of around 17 yielded peptides as the major fraction with a small fraction of partially hydrolysed proteins which is

believed to have fish growth and immune enhancing effects. A series of salmonid feeding studies demonstrated that partly hydrolysed fish protein outperformed fully hydrolysed one (Hardy and others 1983, Stone and others 1989). In the study by Kolkovski and Tandler (2000), freeze-dried squid powder was fully hydrolysed with trypsin and pancreatin where hydrolysate was not as effective as freeze-dried squid protein. It was stressed that an optimum growth response requires a balanced mix of proteins, peptides and free amino acids (Ocean Bioresource Inc., 2002).

Feed attractability and conversion of squid hydrolysate as well as survival rate on trout fingerlings and salmon starters and juveniles revealed that 2-hr squid hydrolysate showed the stronger attractability (21 out of 25 fish) than control (2.5/25), 0 hr-hydrolysate (10.5/25) and 3 hr-hydrolysate (10/25). This may be attributed to increases in attractant free amino acids, gly, ala, and val by 236, 173, and 229%, respectively, in 2 hr hydrolysis, and the presence of high level of feed stimulant betaine. In Atlantic salmon juvenile feeding, diets were prepared with fish meal replacement at 0, 5 and 10% on a protein weight basis. A higher survival rate (77.5% over 65% control) of the diet with 10%-squid hydrolysate replacement, and a higher feed efficiency ratio (1.62 ± 0.11 over 1.34 ± 0.02 control) with 5% replacement were observed. The effect of squid hydrolysate as an attractant and growth stimulation on Atlantic salmon starters was studied using a commercial salmon starter diet spray-coated with 5% and 10% (on a diet weight basis) of liquid squid hydrolysate and oil mixture (8:2). Upon 7-week feeding salmon sacfries (50 fish per 110 gal aquarium), the food conversion ratio (FCR) and specific growth rate (SGR) of the diet coated with 5% of squid hydrolysate were 0.96 and 2.77, respectively, compared to control (1.12 and 2.37).

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L 75. PLANKTON AS FEED RAW MATERIAL

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Abstract

The utilisation of zooplankton from the oceans may in the future give an important contribution to the increasing demand for marine feed materials for the aquaculture industry. The utilisation depends on the resources, catch technology and regulations. We expect that large scale fishing of zooplankton will be carried out by efficient ocean going fishing vessels which deliver their catch to land based plants for processing. This will require stabilising the raw material on board to prevent deterioration in quality.

The aim of the present work is to strengthen the knowledge base of zooplankton with regard to optimal utilisation, and to develop methods for efficient handling of the raw material on board.

The biomass and size, 20 – 50 mm, make the krill species *Meganyctiphanes norwegicus* and *Thysanoessa inermis*, and the amphipod *Themisto libellula* the most likely candidates for exploitation. The fat content in these species are low, 2 – 5 g/100 g, but the krill has an added value of astaxanthin, 20 – 25 mg/kg.

Based on odour, the limit of storage for use as feed raw material is 10 days at 0 °C. The krill species have around 1.4 g TMAO-N/kg, but despite high amounts of TMA in the spoiled product, the odour is characterised by sulphur compounds. There is also a significant formation of cadaverine, and high autolytic activity. The amphipod has around 0.6 g TMAO-N/kg, little formation of cadaverine, and lower autolytic activity.

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L 76. NUTRITIONAL QUALITY OF FISH MEALS MADE FROM BY-PRODUCTS OF THE ALASKA FISHING INDUSTRY IN DIETS FOR PACIFIC WHITE SHRIMP (*LITOPENAEUS VANNAMEI*)

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Abstract

We analyzed the nutritional quality of a variety of commercially available fish meals in obtaining baseline data, as part of an ongoing project with the principal aim of increasing the value of by-products derived from current fish processing methods in Alaska USA. Ten commercially available meals made from fish (primarily pollock) harvested in Alaska waters were obtained. These meals were analyzed for chemical composition and sent to the testing facilities at the Oceanic Institute in Hawaii. Pelleted feeds were made containing these meals in complete replacement of a low-temperature processed Norwegian fish meal, which was used as a control. Pacific white shrimp were obtained from the Oceanic Institute hatchery and reared in forty-two fiberglass containers (1,300 L water). These tanks were filled with seawater initially, and maintained under a zero-water exchange regime (only freshwater was added to make up for evaporation). These feeds were fed using a standard feed table to triplicate tanks of fish for a period of ten weeks. At the end of the trial, the growth data were subjected to analysis of variance procedures (with an error rate of 5%) and differences in means were assessed by Tukey's multiple means comparison test.

The final body weight of the shrimp was approximately ten times the initial weight over the course of the trial with no significant differences between those fed the Alaskan meals and the control. The feed efficiency data followed the pattern of the weight data, indicating that the nutritional quality of these fish meals was indistinguishable from that of the control. Survival exceeded 90% in this trial.

It is concluded that commercially available fish meals from Alaska compare very favorably with the highest standard in the industry and are clearly of suitable quality for consideration for inclusion in feeds for the shrimp culture industry.

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L 77. DIFFERENT FACTORS INFLUENCING THE TEXTURAL PROPERTIES IN THE FILET OF ATLANTIC SALMON *SALMO SALAR*

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Abstract

The Norwegian salmon farming industry uses several smolt production strategies, and this allows the transfer of smolt to seawater almost the whole year around. Fish that are released during spring and autumn will differ in growth pattern during their first year in seawater, as a result of exposure to different water temperatures and photoperiods. This paper will focus on how differences in environmental conditions and fish size influence the textural properties in the filet of Atlantic salmon *Salmo salar*.

Farmed Atlantic salmon derived from three of the smolt production strategies commonly used by the Norwegian aquaculture industry, were sampled during the sea water growth period and assessments of texture were made over a period of approximatly 2 years. Group 1 fish were one year old, 'large graded' smolt, transferred to seawater in April after 16 months in fresh water. Group 2 fish were one year old, 'middle graded' smolt, transferred to seawater in June after 16 months in fresh water. Group 3 fish were underyearling, 'large graded' smolt, transferred to seawater in September after 9 months in freshwater. Four days after each sampling the fish were filleted and the texture was assessed on whole fillet using a Texture analyser (TA-XT2).

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L 78. THE INFLUENCE OF DIFFERENT FEEDING STRATEGIES ON THE FLESH QUALITY OF FARMED ATLANTIC COD (GADUS MORHUA).

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Abstract

It is believed that the feed availability in the ocean influences significantly the flesh quality of cod (*Gadus morhua*). Fillets from cod during abundant feeding in late spring and early summer have shown to be prone to gaping and sometimes the gaping is so serious that the fillets can not be sold as expensive high quality fresh or frozen produce rather as less expensive frozen blocks Farmed cod is usually fed to satiation every day, which may induce gaping problems. To evaluate this Atlantic cod of average weight of 600 g, hatched and raised in an aquaculture station were grown for a year and fed different amount of feed. One group of cod was fed to satiation, a second one on 75% and a third one on 50% of the feed amount that was needed to feed to satiation. The aim of the study was to find out if different feeding strategies would influence the flesh quality of farmed cod.

The weight difference between groups at the end of the experiment was significant. pH measurement showed increasing value with decreasing feeding. Water and protein compensated each other i.e. with decreasing feeding more water and less protein was in the musculature. Instrumental texture measurement on raw fish showed increasing hardness with less feeding but opposite for cooked samples showing decreased hardness with less feeding, which was also the result from sensory analysis. When the cod fed to satiation was processed right after rigor there was no or very little gaping but when the fish had been stored whole in ice for a week pronounced gaping had occurred.

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L 79. QUALITY CHANGES DURING STARVATION OF FARMED AND WILD COD

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Abstract

Farmed cod (*Gadus morhua*) has been found to have a very large liver, low post rigor pH and low water content in the muscle tissue. Especially the low pH implies a different sensory quality compared with wild cod. It has also been claimed that the low pH makes the farmed cod unsuitable for freeze storage.

To investigate how the time of starvation affected the post rigor pH in the muscle tissue, farmed and wild cod were starved during 15 weeks from October to January 2003.

The farmed cod had a high condition factor (0.88) in the beginning of the experiment. The condition factor remained unchanged for 6 weeks and then slowly decreased. The condition factor for the wild was about 10% lower, but was generally following the same trends as the farmed ones.

The liver index was in the beginning of the experiment about three times the normal values in wild cod. The liver index remained stable for 6 weeks, and then it decreased slowly. After 15 weeks of starvation the liver index in farmed cod were still about two times the normal values in wild cod.

The pH was low for the farmed cod during the whole experiment. After 15 weeks of starvation the mean muscle pH remained at 6.4, compared to normal pH of 6.7-6.8. Therefore, starving of cod with a high liver index will not change pH to higher values. The mean water content in the muscle was below 79% during the first 6 weeks and remained below 80% the following 7 weeks. Normal water content in wild cod is between 81-82%. The experiment showed, however, that cod could be starved for at least 6 weeks without loosing muscle weight.

Materials and Methods

Wild cod (*Gadus morhua)* caught outside Bodø September 2001, was fed with dry feed (Dana Feed) until 7th October 2002, when the starvation period started. After one week 10 fish was killed and analysed. With two to three week's intervals new samples were taken, until no more fish remained at the 24th of January 2003. A new batch of wild cod was caught from approximately the same fishing area the 6th November 2002, and stored live in a tank. Samples were taken from the wild population in the same manner as for the fed cod. Only fishes with a gutted weight between 1 to 5 kg were used.

After killing, the fish was bled, and the length in cm and total weight in g was measured. The fish were gutted and the weight of the fish, liver and gonad were measured in g. The fish was stored on ice for four days to pass rigor and then filleted. The fillets were minced in a food processor for analysis of pH and water content.

Condition factor = gutted weight/ length^3 *100
Liver index = liver weight/gutted weight *100
Gonad index = gonad weight/gutted weight *100

pH was measured by inserting a pH electrode (Radiometer pHC2401) directly into the minced muscle tissue.
Water content was measured as weight loss after drying at 104ºC over night.
Principal component analysis was performed using the "Unscrambler" program (Camo Process AS).

Results and Discussion

Table I shows the mean values for starved farmed cod, and table II the mean values for wild cod. During the first three months of starvation the farmed samples were on the average larger than the wild ones. The condition factor was about 10% higher for the farmed cod than for the wild ones. The tables also show that the condition factor remained rather stable in both population during the first two months of starvation and then slowly decreased.

The liver index was three times higher in the farmed group compared with the wild ones. The liver index in the farmed group remained stable during the first six weeks, and then it decreased slowly, in despite of that these cods have started to develop gonads. It also looks like the farmed cod develops gonads earlier than the wild ones.

The post rigor pH remained low (6.2-6.4) during the whole experiment for the farmed cod. After 15 weeks of starvation the mean pH in this group was still found to be below 6.4. The pH in the wild population (6.55) was somewhat lower than expected[1,2] for wild cod in November. After nine weeks of starvation the pH has raised to expected values (6.7–6.8). The mean water content in the muscle was below 79% during the first 6 weeks and remained below 80% during the following 7 weeks. The water content in the wild population was normal (81.3%) in the beginning of the starvation period and raised 1% during the starvation period.

Principal component analysis confirmed the trends, that a well-fed cod will be different from a wild cod even after a three to four months starvation time. The variables condition factor, liver index, pH and water content separates the farmed and the wild cod in two groups and the variables has approximately the same influence on the model.

Table I: Mean biological and chemical values in farmed cod slaughtered after different starvation times in weeks.

Starving time	1 w	4 w	6 w	8 w	10 w	13 w	15 w
Length (cm)	62.2	67.0	64.5	65.8	65.6	67.0	58.4
Total weight (g)	2589	3383	2968	3083	2690	3094	1774
Condition factor	0.88	0.90	0.89	0.84	0.77	0.80	0.71
Liver index	12.7	13.8	13.2	10.8	9.7	11.7	9.4
Gonad index	2.4	3.8	6.1	5.1	10.4	8.9	8.9
pH	6.18	6.30	6.25	6.26	6.17	6.36	6.37
% water	79.0	78.2	78.9	79.5	79.3		79.8

Table II: Mean biological and chemical values in wild cod slaughtered after different starvation times in weeks.

Starving time	4 w	9 w	11 w	13 w	15 w
Length (cm)	58.1	54.7	56.1	58.8	63.4
Total weight (g)	1686	1457	1369	1589	1805
Condition factor	0.76	0.80	0.69	0.68	0.63
Liver index	4.0	3.2	2.2	2.9	2.6
Gonad index	2.7	4.8	4.1	6.4	2.8
pH	6.55	6.78	6.84	6.89	6.80
% water	81.3		82.1	82.7	82.3

Conclusions

The results from this experiment show that starvation of farmed cod is not the way to go, for producing a cod more similar to the wild one. In spite of almost four months starvation time the post rigor muscle pH remained low and the fish still has the characteristics of a farmed cod. If possible the feed and feeding regime must be adjusted during the production period, so the fish will produce more muscle tissue and less liver and glycogen deposits in the muscle tissue. During the winter the cod could be starved for at least six weeks before it starts to loose muscle weight.

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L 80. EFFECT OF PRE SLAUGHTER STRESS ON MUSCLE PROTEIN EXPRESSION IN ATLANTIC SALMON.

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Abstract

Crowding and handling is one among many factors that causes stress in fish and there exists a clear relationship between stress prior to slaughter, and a reduced product quality. The Main effects of stress in Atlantic salmon are on development of *rigor mortis* and important quality attributes such as texture and fillet gaping. Although stress has been evaluated and measured in several ways, the underlying mechanisms of stress related effects on quality are only poorly understood.

Stress leads to major changes in the physiological state of an organism. The cellular balance is distorted due to changes in hormone balances, regulatory mechanisms and metabolic pathways due to altered protein expression. Some proteins display an enhanced expression whereas others are down regulated by stress.

The purpose of the present investigation was to evaluate stress responses in Atlantic salmon muscle by studying protein expression, to extract information on which of the expressed proteins in the muscle change with stress, and to relate this to fillet quality.

An unstressed and a highly stressed group of salmon were compared. At slaughter blood and muscle samples were taken for analysis of cortisol, lactate, glucose, pH and protein expression (proteome analysis). During the succeeding ice storage *rigor mortis*, texture and fillet gaping were measured.

Image analysis of 2-dimensional gels revealed 14 proteins that were expressed at different levels in the two groups. The possible relation between these proteins and dissimilar quality in unstressed and stressed salmon will be discussed.

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L 81. THE EFFECT OF SLAUGHTER METHODS ON EUROPEAN SEA BASS (*DICENTRARCHUS LABRAX)* BEHAVIOUR, RIGOR ONSET, PLASMATIC AND TISSUE STRESS INDEXES AND QUALITY

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Abstract

The aim of the research was to find a practical low stressing slaughtering method and the best quality of small warm species like European sea bass. Several subsequent experiments were carried out in an experimental recirculating system on 300-600 g sea bass, stunned and slaughtered by: 1:2 ice in water (WI), ice-water mixture saturated with N2 (WI-N2) or 40% N2 and 60% CO2 (WI- N2 CO2), asphyxia (AS), CO2 narcosis (AC), electrical stunning (EL), spiking (SP) and knocking (KN). All stunning methods were followed by 30' in ice covering. Fish behaviour, plasmatic and tissue stress indexes, rigor onset and quality involution at 1°C with ice covering by EU scheme were studied. WI slaughter method gave constant results, resulting not particularly stressful, expecially when compared with AS. AC and EL gave less constant results, while SP and KN gave rapid death when correctly performed, but they are not practical methods for this species. The use of gases during fish live chilling was able to shorten death time.

Introduction

Fish slaughtering without any avoidable stress is an ethic requirement at consumer and producer level that also influences product quality. Slaughter methods for marine fish and their impact on fish stress reaction, evolution of *post mortem* biochemical processes and quality of the final product were object of few investigations, especially for Mediterranean species widely consumed in Italy such as European sea bass (*Dicentrarchus labrax*). The aim of this research was to fill this gap trying a practical low stressing slaughter method giving the best quality of fish. It was evaluated the stressing effects on sea bass behaviour and on some stress and quality indicators of: 1) the most used slaughter methods in European fish farms such as asphyxia, cold stunning in ice-water mixture, spiking, knocking, CO_2 narcosis, and electrical stunning; 2) other stunning methods such as gas addition (N_2 and N_2-CO_2) to ice-water slurry, to try a stunning time shortage and an early death.

Materials and methods

Four different experiments were carried out using 300-600 g sea bass (*Dicentrarchus labrax*), reared in Tuscany farms. 10 days before each trial fish were transferred by a van to the research recirculating system, subdivided into 400 l tanks containing sea water (23°C temperature, 7 ppm DO, 24‰ salinity, 14 kg/m^3 density) and let to recover. Water ice mixture 1:2 (WI - normally utilised for sea bass by Italian aquaculture farms), asphyxia (AS), narcosis in CO_2 saturated water (AC), electrical stunning (EL) using Fishkill EG10002 Scubla Aquaculture, Remanzacco, UD, spiking (SP), knocking (KN), ice-water mixture saturated with N_2 (WI-N_2) or with 40% N_2 and 60% CO_2 (WI- N_2 CO_2) were compared, subdivided as follows: trial 1: WI, AS, KN, AC, EL (n. 29 fish, 535±112.3 g b.w.); trial 2: WI, AS, KN, AC, EL, SP (n. 35 fish, 383 ±81.4 g b.w.); trial 3: WI, AS, AC, EL, SP (n. 85 fish, 321 ±82.8 g b.w.); trial 4: WI, SP, WI-N_2, WI- N_2 CO_2 (n. 44 fish, 493 ±97.3 g b.w.). Stunning methods were followed by 30' in ice covering. Parameters evaluated: fish behaviour, death times, hematic parameters - glucose, lactate, cortisol and hematocrit - on blood collected from caudal vein at death; eye liquor, dorsal and caudal pH (pHe, pHd, pHc) and muscle lactic acid, ATP and related catabolites (ADP, AMP, IMP, inosine, hypoxanthine) at death and in the first 24 hours after death, rigor index (RI%) at 3, 6, 9 and 24 hours after death; the quality involution at 1°C with ice covering following the EU scheme (Rule 2406/96 EEC) was evaluated daily by a trained panel of 5 judges, until the fish spoilage. Data were analysed by ANOVA (slaughter methods).

Results

Trial 1 - The evaluation of the behaviour during slaughter and death times showed that KN and SP gave a sudden death, only when performed properly. WI stunned fish in about 20', while CO_2 in 8', but fish showed quick violent spasms. Different voltage stunning were tried for EL fish. 24V for 2' caused immediate loss of consciousness in nearly all fish without causing carcass damage. AS caused violent reactions in the first 3' and a complete loss of movements only after more than 1 h.

At 3 h after death AS and EL had RI% significantly ($p<0.05$) higher (94% and 74%) than KN, WI and AC (30%, 19% and 2% respectively) reaching full rigor at 6 h after death. WI fish showed a higher ($p<0.05$) pH at death at dorsal, caudal and ocular level, while AS showed the lowest dorsal pH. In agreement to these results, ATP level at death was higher in WI and AC fish (3.15 and 2.85 µmol/g) than EL and AS fish (0.22 and 0.17 µmol/g),

while KN showed an intermediate value (0.90µmol/g). Consequently, IMP value was lower in WI and AC. At 24 h after death only inosine showed significantly lower levels of AC, WI and KN (0.43, 0.47 and 0.51 µmol/g respectively) in respect to EL and AS (0.73 and 0.74 µmol/g).

Trial 2 - AS caused higher values of glycemia and lactate, while WI showed the lowest level of cortisol. Full rigor was reached at 3h (AS), 6h (EL) or about 9h (SP, KN, WI, AC) after death. At 3h WI and SP showed lower ($p<0.05$) RI% values (45% and 60%) than AS, AC and KN (100%, 97% and 85%). Also in this trial WI presented the highest dorsal pH at death and AS the lowest. At death, ATP levels were higher in KN and SP (2.11 and 1.97 µmol/g) and lower in AS and AC (0.026 and 0.30 µmol/g). WI and EL showed intermediate values (1.23 and 1.81 µmol/g). Consequently, IMP values resulted lower in SP (6.28 µmol/g) and higher in AS (9.69 µmol/g) which also showed the highest values ($p<0.05$) of inosine both at death and at 24 h from death. The shelf life resulted of about 10 days for WI, one day shorter for SP and KN, and 8 days for EL, AC and AS.

Trial 3 - The AS and EL caused the highest ($p<0.05$) hematocrit levels (51 and 49% *vs* 36, 34 and 26% for WI, AC and SP, respectively). As regards plasma cortisol, the EL fish had the highest ($p<0.05$) concentration (112 ng/ml) and, together with AS fish, the highest plasma lactate (96.6 and 104 mg/dl), confirming the results obtained for hematocrit. The SP fish showed the lowest plasma lactate (33 mg/dl), while the AC and WI fish had intermediate levels (47.8 and 62.8 mg/dl). Also as regards glycemia, AS and EL (251 and 158 mg/dl) seem to be more stressful than SP, AC, and WI (74, 94, and 117mg/dl). The evolution of dorsal and caudal pH at 0, 3, 6, 9 and 24 h after death did not show clear differences among methods. The highest ocular pH at 0 h after death was measured in SP and WI (7.34 and 7.42), the lowest in AS (6.88) and intermediate in AC (7.16) and EL (7.30). The lowest pHe at 24 h *post mortem* was observed in AS and AC fish (6.88 and 7.16 *vs* 7.30, 7.34 and 7.42 for EL, SP and WI respectively). AS and EL fish had the highest muscle L-lactic acid (42.4 and 36.3 µmol/g), while the WI fish had the lowest one (28.2 µmol/g). SP and AC showed intermediate values. Significant differences at death and at 3, 6, 9 and 24 h after death for ATP and its catabolites resulted only for muscle inosine levels, the highest value of which was found in AS fish at every times.

Trial 4 - Behavioural observations indicated that the use of gas in ice slurry was able to reduce the time of stunning: 16-18' for WI-N_2, and 10' for WI-N_2 CO_2 in comparison with the 20' requested for WI. However the WI-N_2 CO_2 induced an early quickly violent reaction, due to CO_2 toxic effect. Blood parameters did not show significant differences among methods with ice slurry, while cortisol (47.56 ng/ml) and lactate (69.92 mg/dl) were higher for SP fish, showing that in this trial the spiking procedure was not done properly. Full rigor was reached after 10 h in SP fish and after 8 h in the other groups. pHe at death showed the highest ($p<0.05$) values for WI than for WI-N_2 and WI-N_2 CO_2 while SP had intermediate values; at 24 h after death the only significant difference was between WI-N_2 and WI. WI and WI-N_2 showed a 9 days shelf life while WI-N_2 CO_2 and SP a 10 day shelf life. The good quality class lasted 4, 3 ,2 days respectively in SP/WI-N_2 and WI-N_2 CO_2/WI.

Conclusions

Asphyxia always produced the most prolonged agony, a remarkable physical activity, a relevant mobilization of energy reserves with earlier rigor onset and shorter shelf life, especially in comparison to spiking, knocking and live chilling. Live chilling did not result particularly stressful, also in comparison both to spiking and knocking, the fastest and generally less aversive slaughtering methods, and to CO_2 narcosis, which in turn was generally less stressful than AS and EL. The most constant results along the several experiments were found with AS and WI slaughtering methods, the most variable ones by CO_2 narcosis and electric stunning. The use of gases during fish live chilling was able to shorten death time without big differences in stress and quality indicators and to produce a day longer shelf life in WI- N_2CO_2 fish. Further analysis are needed for a deeper evaluation of this aspect, bearing in mind the raise of costs due to the use of gases.

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L 82. DEVELOPMENT OF A HUMANE SLAUGHTER METHOD FOR FARMED AFRICAN CATFISH (*CLARIAS GARIEPINUS)*

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Abstract

Ethical aspects concerning food production, such as protection of the environment and animal welfare, are gaining importance. Although concerns about animal welfare apply primarily to mammals and birds, fish welfare, including at the time of slaughter, is gaining interest. A slaughter method is considered to be humane when consciousness is lost immediately by stunning and lasts until death. When stunning is not immediate it should be applied without avoidable stress.

The objectives of the research were development of a humane slaughter method for farmed African catfish (*Clarias gariepinus*) and to discuss its practicality with processors.

In this study two procedures were compared, namely electrical stunning followed by decapitation and electrical stunning in combination with chilling, using an ice slurry. Electrical stunning was performed with individual fish placed in fresh water.

When 300 V, 10 A, 50 Hz a.c. was applied the characteristics of a general epileptiform insult on the EEG were observed, which indicates loss of consciousness. It appeared that a combination of 300 V for 5 s in combination with decapitation or chilling resulted in an immediate and permanent loss of consciousness. Observation of behaviour after decapitation revealed that muscle cramps were absent. For electrical stunning followed by chilling they were present. At a demonstration for Dutch processors of live African catfish it became clear that in their view the latter procedure can be scaled up for use in practice, as during chilling in an ice slurry the unconscious animals are deslimed.

Introduction

In general, food should be safe, tasty and healthy. However, animal welfare aspects are also of importance. Since the last decade concern about welfare aspects of slaughtering farmed fish has been growing. The reasons for the concern is that evidence, suggesting that fish have a capacity to perceive pain, is increasing. Especially at slaughter, applied techniques may be painful, as most industrial methods do not render fish unconscious without avoidable stress prior to killing (Robb and Kestin, 2002; Van de Vis *et al*., 2003). The current slaughter process, which is used in the Netherlands, for African catfish (*Clarias gariepinus*) consists of live chilling to immobilise them prior to evisceration (Robb and Kestin, 2002). In a previous study it was established that live chilling induces aversion and stress in African catfish (unpublished results). For other fish species such as Atlantic salmon, gilthead seabream and eel it was shown humane slaughter methods could be developed (Van de Vis *et al*. 2003). A slaughter method is considered to be humane when unconsciousness is induced immediately, or without avoidable stress, by stunning and lasts until death.

The objectives of the study were development of a humane slaughter method for farmed African catfish and discuss its practicality with processors.

Materials and Methods

Fish

Prior to the experiment African catfishes (n= 31) with a live weight of in the range of 1000-2200 g were fasted and after that delivered to the laboratory. The catfishes were placed in a tank containing aerated tap water at 24 ^{o}C. The experiment was performed with approximately 10 animals per day. After the experiment the fishes were weighed and gutted to determine the sex.

Registration of EEG

On the day of the experiment the fishes were placed one by one in a special developed restrainer for registration of the EEG. The silver spiked EEG electrodes (6 mm long and 1.5 mm in diameter) were positioned in the holes in the head: one electrode 1 cm to the right and one electrode to the left of the sagittal suture and 4 cm caudal the imaginary line between the eyes. The earth electrode for the EEG was placed subcutaneously caudal to the dorsal fin.

The EEG was recorded during 1 minute before and during 2 minutes immediately after stunning and for 30 s 5 and 10 min after stunning. The recorder used was a DI-151RS serial port data recording module with a WinDaq

Waveform browser (Dataq Instruments, Akron, Ohio, USA). During the stunning itself, the EEG and ECG recordings were interrupted to record the applied voltage and current and their duration (in seconds).

Responses to pain stimuli (i.e. needle scratches applied to the skin of the of tail or after decapitation to the head) in the behaviour as well as on the EEG were registered after stunning. The EEG recordings were analysed for changes in the waveforms, frequency and suppression. The behaviour of the animals was monitored for the occurrence of tonic, clonic cramps, exhaustion and recovery.

Stunning

The stunning equipment consisted of a plexiglas box filled with tap water (500 µS conductivity). The bottom and top plate electrodes measured an area of 648 cm^2 each. The distance between lower and upper plate electrode was 16 cm. A power supply (Stork RMS, Lichtenvoorde, Netherlands) delivered constant voltages at 300 V (50 Hz a.c.). The EEG (electro-encephalogram) and observation of behaviour and responses to pain stimuli were used to assess loss of consciousness in African catfishes.

In a previous study it was observed that electrical stunning did not provoke a permanent loss of consciousness. Therefore, the electrical stunning in combination with bleeding by decapitation was assessed for 15 animals. As alternative to this, electrical stunning in combination with chilling in an ice slurry was studied for 16 animals.

Ethics

The experiments were approved beforehand by a Dutch governmental ethical committee.

Results and Discussion

By applying on average 300 V, 10 A, 50 Hz a.c. individual African catfish in fresh water could be rendered unconscious immediately, as the characteristics of a general epileptiform insult were recorded on the EEG. The electrical current was applied for 5 s to prolong the period of unconsciousness to facilitate performing the next step in the slaughter process, namely decapitation or chilling. The next step is required, as in previous studies it was observed that electrical stunning may not result in a permanent loss of consciousness (Van de Vis *et al*., 2003). For both decapitation or chilling it was observed that on the EEG the epileptiform insult changed into low electrical activity without any responses to the applied pain stimuli. These results clearly show that when the fishes were decapitated or placed in an ice slurry within approx. 60 s and 30 after these steps, respectively, no recovery occurred. Assessment by observation of behaviour revealed rapid eye movements, pathological breathing, movements of barbels and absence of muscle cramps in the body after decapitation. For chilling after electrical stunning no responses to scratches applied to the skin of the tail were observed in behaviour. For the latter method muscle cramps occurred.

Concerning avoiding recovery of the stunned animals, the combination of electrical stunning and decapitation is preferred as the supply of oxygen by the flow of blood to the brains is interrupted permanently in the unconscious animal. A combination of electrical stunning and chilling is humane provided that the animals are bled immediately after removal from the ice slurry.

In a discussion with processors of live African catfish both procedures for stunning were assessed with respect to required throughput, price, absence of downgrading, ease of use and compatibility with existing processing lines. It became clear that for batch-wise stunning currents higher than 60 A will have to be used, which is not practicable for these companies. Therefore, electrical stunning of individual fish is preferred. A required throughput of 1 animal per 3 s is possible, provided that a row of 4-5 stunning boxes is set up in the processing line. No problems with bloodspots, bruises or broken bones were observed. Desliming of stunned African catfish is essential to facilitate filleting. In the view of processors African catfishes are deslimed properly by live chilling for 10-15 min in rotation drum filled with flake ice. Therefore, the processors prefer electrical stunning in combination with chilling with flake ice in a rotating drum followed immediately by bleeding.

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L 83. LIFE CYCLE ASSESSMENT OF FRESHWATER TROUT

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Abstract

In recent years the focus has been on the production and quality of food seen in broader terms, especially the methods of production, the resources used and the impact on the surrounding environment. To document this, a Danish research project financed by the Directorate for Food, Fisheries and Agri Business was started in 1998. The objective was to provide a better understanding and assessment of the resource use and environmental impact in agriculture and aquaculture. The subject chosen for aquaculture was rainbow trout *(Onchorynhus mykiss)*. To be able to make a reliable LCA of this fish for human consumption it was necessary to focus the scope of the analysis. It was restricted to 1) trout raised in freshwater facilities in Denmark at 2) maximum seize of 400 g 3) fish feed with sandeel *(Ammodytes ssp)* as the animal protein and oil resource and 4) excluding the last links in the chain, the retailers and consumers. The results from the LCA on trout will be presented and the difficulties in finding the proper information to make the analysis will be discussed. The primary contribution to the environmental impact comes from the catching of the fishes used to produce the fishmeal and –oil. The impact between different types of aquaculture facilities will be discussed and the future development of organic trout production will be included.

Introduction

There is a growing global interest in the impact on the environment coming from human activities. This has resulted in two huge conferences, one in Kyoto1997 where e.g. the Danish Minister of Environment promised that Denmark should reduce its emission by 25 % in 2010 and a conference in Johannesburg in 2002, where sustainable development was one of the headlines.

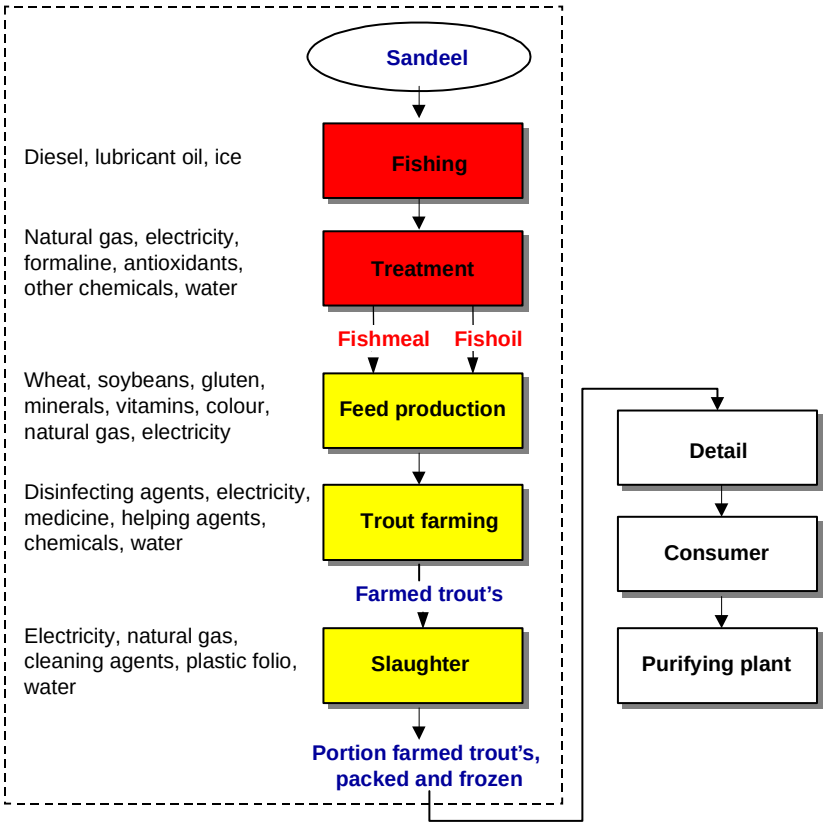

Figure 1: Illustration of the process flow for production of trouts.

There is an increasing demand for exact knowledge of how environment friendly a product is. This interest includes the production and quality of food. Both for the primary agriculture production and for fishery there is a lack of coherent and relevant information, which can form the basis for a life cycle assessment. On this background a project was started in 1999 and the part dealing with production of freshwater trout is presented in this paper.

The reason for choosing aquaculture is that it is analogue to primary agriculture and the data collected could be analysed in a similarly way. (http://www.lcafood.dk/)

Materials and Methods

In this study the environmental impacts from average Danish trout production is compared with environmental impacts from trout production with 0 and 100 % recirculation of water. Methodologically the work is based on life cycle assessment beginning with the sandeel fishing for reduction to fishmeal and -oil used in feed production for trout and ending at the trout slaughterhouse, where the trout's are processed and primarily frozen before distribution to the marked. The process flow with inputs is illustrated in figure 1.

Freshwater trout or rainbow trout belongs to the salmon family. The trout meat is pale brown to almost white. The flake is small and the bones easily removed (Frimondt 1995). Trout raised in farms are usually delivered to filleting and freezing factories for further processing to the consumer.

The present data refer to trout's (200-400 g) produced in Danish trout farms in 2000.

Production of trout's can vary due to local conditions, such as water flow and other hydrological conditions. The importance of different production stages is studied and the trout production is compared with environmental impacts caused from catching of other fish types.

Results and Discussion

Impact on the surrounding environment measured as global warming, acidification and eutrophication from production of freshwater trout is studied in this life cycle assessment.

Figure 2 shows the energy used in the different steps when producing rainbow trout. This study does not including data from transportation between the different process steps. Most of the global warming caused by production of raised rainbow trout originates from the operations for fishing sandeel and the production of fishmeal due to the amount of energy used in these process steps.

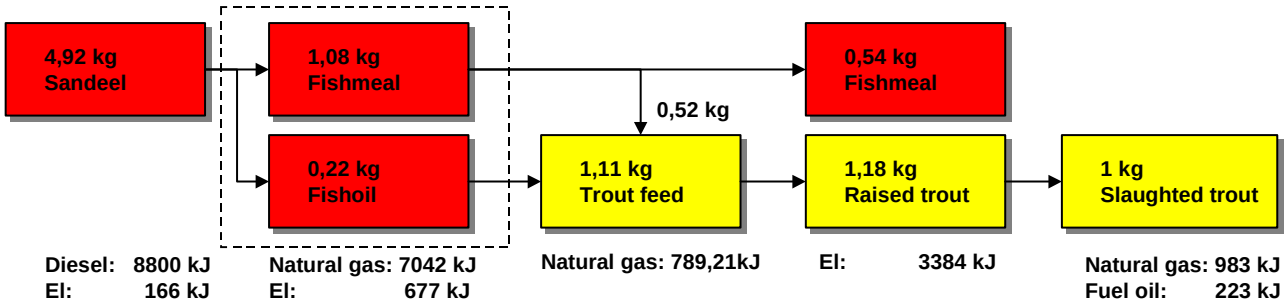

Figure 2: Energy used in the different process steps.

Energy is one of many factors that is resulting in the total environmental impacts from the production of trout.

In figure 3 all the important input and output from the production of trout are included, and the factors global warming, acidification and eutrophication illustrate the environmental impact. Production of trout (0 % recirculation of water), trout (100 % recirculation of water) and normal standard trout is compared. As shown, production of trout by using 100 % recirculation of water contributes more to global warming and acidification than the other production methods. Trout production with 0 % recirculation has the highest impact on the environment, because of eutrophication.

In figure 4 the standard trout production of fillets is compared with production of fillets from wild caught fish cod, flatfish and mackerel and cold-water shrimps (lcafood 2003). This comparation shows, that production of shrimps effects the global warming and acidification much more than production of freshwater rainbow trout in Danish aquaculture. This can be explained by the relative small quantity of shrimps caught, compared with the catch effort using large factory trawlers. The Danish trout production attributes more to the eutrophication than the other fish products.

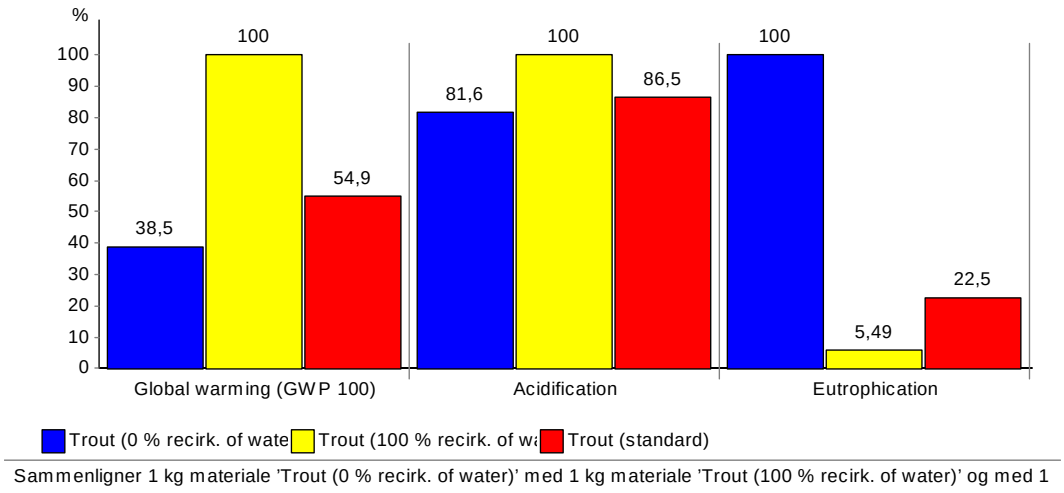

Figure 3: Comparison of trout raised whit different use of water recirculation. *(SigmaPro 2003)*

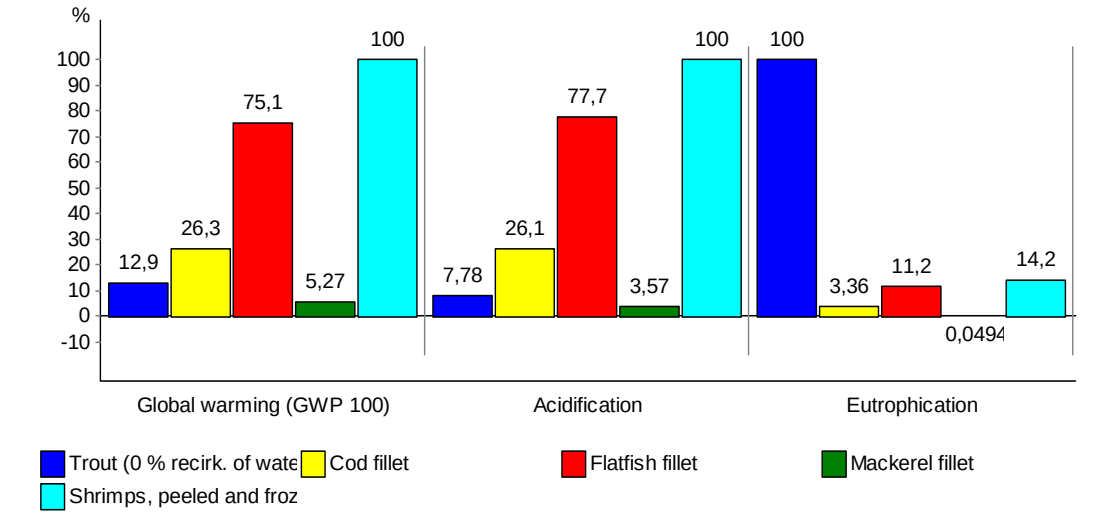

Figure 4: Comparision of trout with different wild fish. *(SigmaPro 2003)*

Conclusion

The study of the production of freshwater trout and following life cycle analysis showed that the highest proportion of emission comes from the catching of the small fish that are used for reduction to fish meal and fish oil.

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L 84. MINIMISATION, RECYCLING AND FINAL TREATMENT OPPORTUNITIES FOR SOLID WASTES FROM FRESH WATER FISH FARMS

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Abstract

Fresh water fish farms, as other industrial activities, generate solid residues, originated basically in the gutting and filleting processes, and occasionally due to accidents, important amounts of dead fish that have to be properly managed. These organic wastes have an important water and oil content. A diagnosis of the Spanish continental aquaculture sector was made in order to study the best alternative to manage the wastes generated by this sector. At the same time, the evaluation of the alternatives for minimisation, recycling and the final treatment of the solid wastes were studied, together with the introduction of the continuous environmental improvement concept in the companies.

The aim of the study is to tackle the waste and environmental issue and to seek a solution to convert the problem into a business opportunity. The study has two main approaches. The first one is reduction on site, by applying Clean Technologies to minimize the waste production at origin and to reduce cost by optimising the use of resources. The second approach deals with the character of the wastes. Different alternatives are being studied for the final use of the generated wastes by transforming them into a sub-product. The alternative studied vary from the transformation of wastes into pet foods to the recuperation of high value products.

Introduction

Fish consumption in Spain has one of the most important rates in the world and aquaculture is the alternative to answer the existing demand for fish products. Nowadays the Spanish trout aquaculture sector is in an expansion phase, and developing towards a clean, and sustainable industry.

The main objectives sought in this project were: to analyse the processes for identification of the main environmental problems; to study the existing alternatives to minimise the generation of wastes and to evaluate the liability of those alternatives; to identify the existing alternatives for the final treatment and management of the wastes and to select the best alternatives for their further implementation, in accordance with the legal and technical requirements to be fulfilled by each type of waste management option.

Materials and Methods

To achieve the above mentioned objectives the project was structured in different phases. The methodology used for each phase was:

- Waste characterisation: For each waste type, the physico-chemical characterization of the waste, their source, the generated quantities and the actual type of waste management is described. This study was done for dead fishes, heads and guts, sludge, paper and corrugated paper, and wooden pallets. The data were collected through questionnaires sent to the participant plants, located in the North, Central-East and Central-West Spain. The plants were selected as representatives for small, medium and large size companies.
- Legal review: The European, Spanish and local legislation was reviewed in order to identify the existing regulations for the different types of wastes and their management.
- Study of minimisation alternatives: Clean Production principles were applied: These principles are: **prevention**, then **reduction**, then **internal recycling**. The implementation of the Clean Production depends on the characteristics of each plant and their environmental issues, therefore a specific implementation plan was applied for each production site. The proposed alternatives are described taking into account the required investments, the achieved benefits, the foreseen savings and the environmental consequences, based on a technical and economical assessment.
- Identification and evaluation of valorisation alternatives: Review of the on-going research projects, bibliographic reviews and technological surveillance regarding added value products and valorisation systems were carried out. The studied alternatives were classified as recycling alternatives, allowing to obtain a product or a benefit, or elimination alternatives. The assessment was done considering legal, technical and economical criteria.

Results and Discussion

Prevention and minimisation alternatives

Clean Production is defined as the implementation of organisational and operational preventive measures to improve the efficiency of the productive processes and to minimise the risk on workers and the possible damage to the environment. The Clean Production aims to avoid the problem, this is to prevent before the problem arouses since it is much easier and cheaper to treat the cause rather than the problem itself.

The identified and developed minimisation measures among others are: personnel training, collecting information about generated wastes, recovery of fish oils, modification of ergonomics of equipments, floor dry-cleaning before using water, implementation of double nets to catch solid wastes in drains, hydraulic improvement of fish tanks, avoiding excess numbers of fish in tanks, negotiation with suppliers, use of returnable packages, separation of wastes at origin.

Recycling, valorisation and treatment alternatives

The elimination and authorised alternatives for dead fish are: incineration, or processing (according to Spanish law RD 2224/93) and further incineration. This are basically applicable to dead fish, since no other treatment is allowed besides of energetic uses.

For the rest of the organic wastes, the possible treatment alternatives are:

Composting: best alternative for managing the sludge (fish excrements), since it is difficult to valorise this product into any other use. To optimise the compost quality it is recommended to mix the sludge with vegetal residues.

Biogas production*:* alternative for dead fish and sludge. It requires a high inversion but the bio-metanization, besides producing energy, has the advantage that the produced residue can be reused for composting purposes.

Other agro uses: Traditionally fish wastes were used as natural fertilizers, but nowadays this measures´ suitability is under question due to sanitary reasons. Nevertheless under the right treatment, fish wastes have a very adequate composition to be used to improve the composition of soils.

Silage: This treatment allows to maintain the fish protein characteristics in excellent conditions. It requires low investments, but the commercialisation of these products is not adequately developed in Spain.

Production of fish meal: there is a wide industrial use of fish meals to treat the fish wastes (head and guts), mainly to produce pet foods. It is the easiest alternative to be implemented, although the logistic of waste collection has to be improved.

High added value product production: Due to the characteristics of the head-gut wastes, a number of high value compounds susceptible of being produced were identified, such as: protein concentrates, fish liver oil, albumin, peptones, amino acids, mink feed, fish jelly, fish leather, pearl essence (cuanine), surimi and marinbeef.

Conclusions

Waste management is not an easy task, mainly due to the remote location of most of the trout producing farms. Therefore, it is very important to reduce the waste at origin for diminishing their management costs at the same time.

This project has allowed to characterise the wastes of trout farming and processing plants and to propose minimisation alternatives such as the separation and selection of the different types of wastes, to control the management of the wastes, and to involve the suppliers as much as possible to reduce the generation of wastes (for example packaging materials).

As a result of the study, the recommended uses for the different wastes are:

- Dead fish: considered as High Risk Material, therefore the alternatives are: to eliminate them as Specific Risk Material, incineration, or production of biogas.
- Heads and guts: the alternatives are: disposal in dumps, production of fish meal for pet foods, production of fish meal for other animals but ruminants. As far as the studied alternatives for the use of the organic wastes generated during the filleting processes, nowadays the most economical solution is the elaboration of fish meal. Other possibilities to obtain added value products were nevertheless identified, but these options need further research and technical and economical feasibility studies. Incineration, production of biogas, and composting are other allowed possibilities, but have low commercial added value.
- Other organic wastes: the alternatives are: disposal in dumps, incineration, composting and production of biogas, being the last one of the best alternatives.

During the project other waste problems were detected: packaging materials, dangerous wastes, water use in the plants, etc…

The implementation of an Environmental Management System was a general recommendation, which will not only contribute to obtain an environmental certification but also to improve the image of each farm and factory and of the sector in general.

Acknowledgements

This project was implemented in the OPAC, Organization of Continental Aquaculture Producers, with the financial support of the Spanish Ministry of Fisheries.

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P 68. COMPARISON OF THE TOTAL LIPID CONTENT AND FATTY ACID PROFILE OF FLESH AMONG WILD, EXTENSIVELY AND INTENSIVELY FARMED SEA BASS (*DICENTRARCHUS LABRAX*)

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Abstract

A study was carried out on the content of lipid in the fillet and the fatty acid composition of 120 sea bass, came from three different environments: SB1 = wild sea bass captured in Italian open seawater; SB2 = reared extensively and caught in valli of the north Adriatic Sea; SB3 = came from an intensive farm where were reared in concrete basins.

At sampling, fish were filleted, after removing skin and subcutaneous adipose panicle, and the dorsal muscle (central part) was analysed.

Total lipid were extracted with chloroform/methanol (2:1) according to the procedure of Folch et al. (1956) and were converted to fatty acid methyl esters following the Christopherson and Glass (1969) methods. A Carlo Erba HRCG5160 with DP 6700 integration system, equipped with a SP 2340 capillary column, was used to determine the fatty acid composition.

Results showed an increase in sea bass flesh lipid content passing from a minimum level observed in SB1 (1.99%) to a maximum rate in SB3 (3.21%). Changes were found in the fatty acid profile with high variability in proportion of saturated, monounsaturated and polyunsaturated fractions among the different groups.

Acknowledgements

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P 69. INFLUENCE OF RESPONSIBLE REARING TECHNIQUES ON FLESH QUALITY AND WELFARE STATUS OF RAINBOW TROUT (*ONCORHYNCHUS MYKISS*)

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Abstract

During 2001-2002 trials were carried out with farmed rainbow trout comparing Experimental groups and Controls, aimed to reduce the lipid content of the muscle and to improve the fatty acid profile of flesh through the reduction of lipid content of daily ration and the stocking density of fish.

The length of fattening phase, starting from 40±20 g b.w., was fixed in 12 months (final mean weight = 350±50 g).

At the end of each fattening cycle, a significant sample of fish of both Experimental and Control groups was analysed to evaluate fatty acid profile, total cholesterol and lipids.

The welfare status was monitored 4 times during the fattening phase by means of haematochemical analysis of blood samples of 5 fish/each replicate of the groups. The parameters considered were: glucose, total cholesterol, triglycerides, CK, FAL, GOT, GPT, LDH, GGT by spectrophotometer using international procedures.

A significant decrease of total lipid content was observed in the flesh of Experimental groups (I cycle: 3.29%; II cycle: 2.66%) compared to Controls (I cycle: 4.43%; II cycle: 3.59%). The fatty acid pattern was markedly affected by the fatty acid composition of the Experimental diet administered. In all the Experimental groups a lower level of saturated and monounsaturated fatty acids and a higher PUFA content were observed compared to Control fish. ω6:ω3 ratio decreased thanks to the increase of polyunsaturated fraction. A significant low concentration of total cholesterol was detected in fish reared with Experimental program.

Introduction

In the last years, the events such as BSE, dioxins and PCB's contamination of feedstuffs used in animal feeding (Hertrampf and Piedad-Pasqual, 2000) stimulated consumers to get a better knowledge about qualitative traits of farmed animals (Kestin and Warriss, 2001). The consequence was an increase of demand of higher quality products obtained with the respect of environment and animal welfare. As for aquaculture, this situation caused a high suspicion towards reared fish so farmers and large scale distribution recognised the need to enhance the safety and nutritional quality of fish promoting guidelines to improve the quality of fish diets, rearing environment and animal welfare.

Trials were carried out with rainbow trout, comparing Experimental groups and Controls, aimed to reduce the lipid content of the muscle and to improve the fatty acid profile of meat through the reduction of lipid content of daily ration and the stocking density of fish.

Materials and Methods

During 2001-2002 trials were carried out with farmed rainbow trout comparing Experimental groups and Controls, with 2 replicates each, reared in concrete raceways, aimed to reduce the lipid content of the muscle and to improve the fatty acid profile of flesh through the reduction of lipid content of daily ration, the stocking density of fish (40 kg/m^3 *vs* 60 kg/m^3).

The highest daily ration of Experimental groups was 0.8% b.w. *vs* 1.2% b.w. of Control.

The length of fattening phase, starting from 40±20 g b.w., was fixed in 12 months (final mean weight = 350±50 g).

At the end of each fattening cycle, a significant sample of fish of both Experimental and Control groups was analysed to evaluate fatty acid profile, total cholesterol and lipids.

Flesh samples were collected from the dorsal muscle (central part) after having removed skin and subcutaneous adipose panicle.

Total lipid were extracted with chloroform/methanol (2:1) according to the procedure of Folch et al. (1956) and were converted to fatty acid methyl esters following the Christopherson and Glass (1969) methods. A Carlo Erba HRCG5160 with DP 6700 integration system, equipped with a SP 2340 capillary column, was used to determine the fatty acid composition. The concentration of total cholesterol was calculated using as internal standard beta-sytosterol.

The welfare status was monitored 4 times during the fattening phase by means of haematochemical analysis of blood samples of 5 fish/each replicate of the groups.

The parameters considered were: glucose, total cholesterol, triglycerides, CK, FAL, GOT, GPT, LDH, GGT by spectrophotometer using international procedures.

Results and Discussion

A significant decrease of total lipid content was observed in the flesh of Experimental groups (I cycle: 3.29%; II cycle: 2.66%) compared to Controls (I cycle: 4.43%; II cycle: 3.59%).

The fatty acid pattern was markedly affected by the fatty acid composition of the Experimental diet administered.

In all the Experimental groups a lower level of saturated and monounsaturated fatty acids and a higher PUFA content were observed compared to Control fish.

ω6:ω3 ratio decreased thanks to the increase of polyunsaturated fraction.

A significant low concentration of total cholesterol was detected in fish reared with Experimental program ranging from the mean values (I cycle: 50.74 mg/100 g; II cycle: 46.67 mg/100 g) to Controls (I cycle: 65.76 mg/100 g; II cycle: 61.86 mg/100 g).

Haematological investigations showed very similar values about total cholesterol and triglycerides that did not differ significantly. Plasma activities of the enzymes examined (creatine kinase, FAL, GOT, GPT, lactate dehydrogenase, GGT) and glucose showed considerable individual variations in the two groups.

Conclusions

Comparing the Experimental groups and the Controls, it has been possible to ascertain that the reduction of the daily ration, the lipid content of feed and the stocking density had not significant effects on the final mean body weight and, at the same time, had positive effects on the total lipid content and fatty acid profile of the fillet.

This work was carried out in accord with fish farm companies after an Italian Great Distribution (G.D.) gave the availability to retire an end-product with high qualitative and safety guarantees, obtained by means of specific controlled farming practices. In this way, it should be possible help the consumers' choice in their buying decision-making.

Acknowledgements

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