Sensory, microbial and chemical effects of a slurry ice system on horse mackerel (*Trachurus trachurus*)

Desired section: Sensory and nutritive qualities of food

Title:

Extended Shelf Life of Horse Mackerel (*Trachurus trachurus*) Chilled in Slurry Ice as Determined by Sensory and Microbiological Analysis.

Authors:

Óscar Rodríguez ^a, [¿Vanesa?](#), Santiago P. Aubourg ^b, Jorge Barros-Velázquez ^a

Affiliations:

^a *Department of Analytical Chemistry, Nutrition and Food Science, College of Veterinary Sciences, University of Santiago de Compostela, E-27002 Lugo, Spain*

^b *Department of Seafood Chemistry and Technology, Institute for Marine Research, Higher Council for Scientific Research (IIM-CSIC), C/ Eduardo Cabello 6, E-36208 Vigo, Spain*

Corresponding author: Professor Jorge Barros-Velázquez, Department of Analytical Chemistry, Nutrition and Food Science, College of Veterinary Sciences, University of Santiago de Compostela, E-27002 Lugo, Spain. Tel.: +34-600-942-264; fax: +34-982-252195; E-mail address: jbarros@lugo.usc.es

Short title: Extended shelf life of horse mackerel in slurry ice

[Horse mackerel shelf-life in slurry ice](#)

ABSTRACT

Slurry ice, a biphasic system consisting of small spherical ice crystals surrounded by seawater, was evaluated in parallel to flake ice for the storage of horse mackerel (*Trachurus trachurus*). Storage in slurry ice implied a significant enhancement of the shelf life (5d for flake ice to 15d for slurry ice), better control of pH value, and lower counts of total aerobes, proteolytic and lipolytic bacteria, these reaching average differences between batches of 2, 1.43 and 1.98 log units, respectively, after 8 d of storage. Storage in slurry ice also implied significantly slower formation of total volatile base-nitrogen and trimethylamine after 8 d of storage. *Staphylococcus xylosus* and *Proteus penneri* were identified as the leading proteolytic and lipolytic organisms in horse mackerel muscle. Storage of horse mackerel in slurry ice enhances the shelf life of this medium-fat fish species through a better maintenance of sensory and microbiological quality.

Key Words: Horse mackerel; Slurry ice; Shelf life; Sensory quality; Microbiological activity; Proteolysis; Lipolysis.

1 INTRODUCTION

2
3 Aquatic food products deteriorate rapidly due to the joint action of microbiological,
4 enzyme and chemical spoilage mechanisms (Pigott and Tucker 1987; Hsieh and
5 Kinsella 1989). During chilled storage damage to the fish is slowed down but not
6 prevented, this leading to losses in sensory quality and nutritional value (Whittle and
7 others 1990). While traditional chilling has involved the use of flake ice (Nunes and
8 others 1992) or refrigerated seawater (Kraus 1992), more recently, slurry ice –also
9 known as fluid ice, slush ice, liquid ice or flow ice– has been introduced as a promising
10 technique for the preservation of fish products at subzero temperature.

11 Slurry ice can be defined as a biphasic system consisting of small spherical ice
12 crystals suspended in iced water at a temperature slightly above the initial freezing point
13 of fish (0°C to -2°C). Among the main advantages of slurry ice, two should be
14 highlighted: its faster chilling rate –due to its higher heat-exchange capacity–, and the
15 limited physical damage that causes to fish food products –due to the spherical
16 geometry of its microscopic ice crystals–. Other advantages of slurry ice derive from its
17 complete coverage of the fish surface, which affords a better protection of the fish
18 surface with respect to oxidation and dehydration events. Slurry ice can also be pumped,
19 this guaranteeing a more hygienic handling of the fish products, and may be combined
20 with other agents, such as ozone, to achieve an antiseptic surface effect, or melanosis
21 inhibitors, to prevent browning reactions in shellfish (Huidobro and others 2002).

22 Chapman (1990) reported a better maintenance of quality of finfish stored on-board
23 in slurry ice as compared with other chilling methods, a result similar to that found for
24 the on-board storage of albacore tuna by other authors (Price and others 1991). Harada
25 (1991) also underlined the advantages of slurry ice as a pre-cooling method for fish. The
26 scientific literature recently accounts for the use of slurry ice systems for the storage of
27 Australian prawns (Chinivasagam and others 1998), and shrimp (Huidobro and others
28 2002). Other authors have also reported that slurry ice represents a good slaughter
29 method to sacrifice and store farmed seabream (Huidobro and others 2001).

30 Horse mackerel is a medium-fat species abundant in Northeast Atlantic (FAO 1998;
31 Aubourg and Ugliano 2002) and has a potential role in the prevention of heart disease
32 due of its high content in PUFAs (n-3) not synthesized by humans. Horse mackerel has
33 not been extensively utilized as raw material for the fish industry in the past, but is
34 deserving an increasing attention from fish technologists, being currently considered as
35 an infra-utilized fish species with a high commercial potential as an effective functional
36 food (García and others 1996; Tabara and others 1998). The minimal seasonal variation
37 of the horse mackerel lipids was reported by Bandarra and others (2001), who also
38 underlined the nutritional interest of this fish species as an important yr-round source of
39 lipids of dietary importance. Previous research carried out on chilling storage work
40 reports biochemical analyses (amine formation and lipid damage) (Aubourg, 2001) and
41 physico-chemical parameters (Monteagudo-Torres and others 2002), although
42 microbiological parameters were not considered.

43 In this work we have applied an advanced slurry ice system to the storage of horse
44 mackerel (*Trachurus trachurus*) during 22 d, and compared with a control batch stored
45 in parallel in conventional flake ice. With a view to investigating the shelf life of horse
46 mackerel, here the effects of storage of horse mackerel in slurry ice on sensory and
47 microbiological quality were investigated during 22 d. In addition, the isolation and
48 identification of major bacteria involved in the proteolytic and lipolytic breakdown of
49 horse mackerel muscle was also undertaken.

MATERIALS AND METHODS

Slurry ice and flake ice systems

A slurry ice prototype (FLO-ICE, Kinarca S.A.U., Vigo, Spain) was used in the present work. The composition of the slurry ice binary mixture was 40% ice and 60% water, prepared from filtered seawater (salinity: 3.3%). The temperature of the slurry ice mixture was -1.5°C. Flake ice was prepared with an Icematic F100 Compact device (CASTELMAC SPA, Castelfranco, Italy).

Fish material, processing and sampling

Specimens of horse mackerel (*Trachurus trachurus*) were caught during the day at a local fishing bank close to Northwestern Spain and kept on ice until they arrived at our laboratory. The fish specimens were neither headed nor gutted. The length of the specimens was in the range of 16-21 cm; the weight was in the range of 230-270 g. The fish specimens were placed in either slurry or flake ice at a fish:ice proportion of 1:1, and stored for up to 22 d in a refrigerated room at 2°C. When required, the flake ice and the slurry ice mixture were renewed.

For each chilling treatment, three different batches were used and studied separately along the whole experimental period. Samples were taken from each batch on days 0, 2, 5, 8, 12, 15, 19 and 22. Once the intact specimens had been subjected to sensory analyses, the white muscle was separated and used for microbiological and chemical analyses; all analyses were performed in triplicate.

Sensory analyses

Sensory analyses were conducted by a taste panel consisting of five experienced judges, according to the guidelines presented in Table 1 (DOCE 1989). Four categories were ranked: highest quality (E), good quality (A), fair quality (B) and unacceptable (C). Sensory assessment of the fish included the following parameters: skin, external odor, gills, eyes, consistency and flesh odour.

Microbiological analyses

Samples of 25 g of fish muscle were dissected aseptically from chilled horse mackerel specimens, mixed with 225 ml of 0.1% peptone water, and homogenised in a stomacher (Seward Medical, London, UK) as previously described (Ben-Gigirey and others 1998, 1999). For assays at abusive temperatures, whole fish fillets were placed inside sterile bags and kept at 30°C for 3 d before the fish extracts were prepared. In all cases, serial dilutions from the microbial extracts were prepared in 0.1% peptone water. Total aerobes were investigated in plate count agar (PCA, Oxoid Ltd., London, UK) after incubation at 31°C for 72 h. Anaerobes were investigated in the same way, except that an anaerobic atmosphere kit (Oxoid) was placed together with the plates inside the anaerobiosis jar. Lactose-fermenting *Enterobacteriaceae* (coliforms) were investigated in Violet Red Bile Agar (VRBA medium, Merck, Darmstadt, Germany) after incubation at 30°C±1°C for 24±2 h, as recommended by the manufacturer (Merck Microbiology Manual, 2002).

Microorganisms exhibiting a proteolytic phenotype were investigated in casein-agar medium (30°C/48 h) (Phaff and others 1994), as previously described (Ben-Gigirey and others 2000). Bacterial colonies exhibiting a lipolytic phenotype were detected in tributyrine-agar medium, as described elsewhere (Ben-Gigirey and others 2000).

1 **Table 1 – Scale employed for evaluating the freshness of horse mackerel.**

Attribute	Highest quality (E)	Good quality (A)	Fair quality (B)	Unacceptable (C)
Skin	Very intense pigmentation; transparent mucus	Milky mucus; insignificant pigmentation losses	Slightly greyish mucus; pigmentation without shine	Widely opaque mucus; important pigmentation losses
External odour	Sharply seaweedy and shellfish smell	Weakly seaweedy and shellfish smell	Incipiently putrid and rancid	Putrid and rancid
Gills	Brightly red; without odour; lamina perfectly separated	Rose coloured; without odour; lamina adhered in groups	Slightly pale; incipient fishy odour; lamina adhered in groups	Grey-yellowish colour; intense ammonia odour; lamina totally adhered
Eyes	Convex; transparent cornea; bright and black pupil	Convex and slightly sunken; slightly opalescent cornea; black and cloudy pupil	Flat; opalescent cornea; opaque pupil	Concave and milky cornea; Internal organs blurred
Consistency	Presence or partial disappearance of rigor mortis symptoms	Firm and elastic; pressure signs disappear immediately and completely	Presence of mechanical signs; elasticity notably reduced	Important shape changes due to mechanical factors
Flesh odour	Sharply seaweedy and shellfish smell	Weakly seaweedy and shellfish smell	Incipiently putrid and rancid	Putrid and rancid

Routine microbiological tests included the investigation of colony morphology, cell morphology, motility, Gram stain, and production of cytochrome oxidase and catalase, as described elsewhere (Rodríguez and others 2003a). The identification of major proteolytic and lipolytic bacterial strains was accomplished using miniaturized biochemical tests: API 20 E and API 20 NE for Gram-negative microorganisms, and API 50CH and API STAPH for Gram-positive microorganisms, all of them from BioMérieux (Marcy L'Etoile, France). The results of the identification tests were interpreted using the APILAB PLUS software (BioMérieux). The enzymic profiles of the proteolytic and lipolytic bacterial isolates were further characterized using the API ZYM system (BioMérieux).

Chemical analyses

The evolution of pH values along the storage time was carried out using a 6-mm diameter insertion electrode (Crison, Barcelona, Spain). Total volatile base-nitrogen (TVB-N) values were measured according to Aubourg and others (1997). On it, fish muscle (10 g) is extracted with perchloric acid (6%) and made up to 50 ml, the TVB-N content being determined –after steam-distillation of the acid extracts rendered alkaline to pH 13 with NaOH (20%)– by titration of the distillate with 10 mM hydrochloric acid. The results are expressed as mg TVB-N/100 g muscle. Trimethylamine-nitrogen (TMA-N) values were obtained by the Tozawa and others (1971) method, which involves the preparation of a 5% trichloroacetic acid extract of fish muscle (10 g/25 ml) and reaction with picric acid. Data are expressed as mg TMA-N/100 g muscle.

Statistical analyses

Bacterial counts were transformed into log CFU/g before subjecting to statistical analyses. Data from the different chemical measurements were subjected to one-way analysis of variance; comparison of means was performed using a least-significant-difference (LSD) method (Statsoft 1994). The SPSS 11.5 software for Windows (SPSS Inc., Chicago, IL) was also used to explore the statistical significance of the results obtained, this including multivariate contrasts and multiple comparisons by the Scheffé and Tukey tests. A confidence interval at the 95 % level ($p < 0.05$) was considered in all cases.

RESULTS AND DISCUSSION

Sensory analyses

According to the results of the sensory analyses (Table 2), horse mackerel stored in slurry ice maintained good quality (E and A categories) up to day 8, while the counterpart batch stored in flake ice only maintained such quality up to day 2. As storage time progressed, sensory quality decreased and by day 8 (flake ice batch) and day 19 (slurry ice batch), the specimens were no longer acceptable. The external features that limited the acceptability of the flake ice batch were: the external odour, the flesh odour and the gills. The shelf life of horse mackerel stored in flake ice determined in this study agreed with previous works carried out with this fish species (Simeonidou and others 1997; Aubourg 2001) and with other small fish species such as sardine (Nunes and others 1992) and mackerel (Bennour and others 1991). It should be remarked the considerable increase of shelf life obtained as a consequence of the use of slurry ice employment, the horse mackerel specimens keeping an acceptable quality at least until day 15. A similar enhancement of shelf life as a consequence of the use of slurry ice has been recently reported for lean fish species such as turbot (Rodríguez and others 2003b) or hake (Rodríguez and others 2003c).

Quantitative microbiological analyses

Figures 1, 2 and 3 display the most relevant results concerning microbial growth in horse mackerel stored in either slurry ice or flake ice. Statistically significant ($p < 0.05$) differences were observed between both batches for aerobes, proteolytic and lipolytic bacteria. In the case of total aerobes, average difference in the counts between batches

1 **Table 2 – Comparative sensory evaluation of horse mackerel batches.^a**

	Slurry ice batch (days of storage)							Flake ice batch (days of storage)						
	2	5	8	12	15	19	22	2	5	8	12	15	19	22
Skin aspect	E	E	A	A	B	C	C	E	A	B	C	C	C	C
External odour	E	A	A	B	B	C	C	E	A	C	C	C	C	C
Gills	E	E	A	B	B	C	C	E	B	C	C	C	C	C
Eyes	E	A	A	B	B	C	C	E	A	B	B	C	C	C
Consistency	E	E	A	A	B	B	B	E	A	B	B	B	C	C
Flesh odour	E	A	A	B	B	C	C	E	A	C	C	C	C	C

2 ^a Freshness categories are as expressed in Table 1. Initial quality at day 0 deserved an “E” score (highest quality) for all parameters.

1 **Figure 1 – Comparative evolution of total aerobes in horse mackerel muscle during**
2 **storage in either slurry ice (□) or flake ice (◇).**

was 2.0 log units on day 8, and this difference even increased to 2.61 log units after 12 d of storage (Figure 1). At both sampling times the flake ice batch had exhibited unacceptable quality according to sensory evaluation, but the slurry ice batch still maintained acceptable quality (Table 2). The total aerobic counts reached levels close to 10^6 CFU/g in the flake ice batch after 8 d of storage, although these numbers were below those considered by other authors to provoke the spoilage of fish stored aerobically (Gram and Huss 1996). Storage of horse mackerel in slurry significantly reduced bacterial growth, this may contributing to the enhanced shelf life determined by sensory evaluation. These results agree with recent works reporting significantly lower bacterial counts in shrimp (Huidobro and others 2002), turbot (Rodríguez and others 2003b), and hake (Rodríguez and others 2003c) stored in slurry ice, as compared with counterpart batches stored in conventional flake ice. A recent work has reported aerobic bacteria counts as high as 10^7 CFU/g in the skin of horse mackerel stored for 7 d at 4°C (Kuda and others 2002). In this sense, the surface wash caused by the liquid phase of the slurry ice together with the subzero temperature achieved with this advanced storage system, may be argued as the two main reasons of the limited bacterial growth found in the muscle of horse mackerel in the slurry ice batch, as compared with the flake ice batch.

Microbial metabolites such as peptides or amino acids derive from protein hydrolysis and may also contribute significantly to undesirable sensory changes in seafood products. It is well known that such modifications in odour, texture and appearance are directly related to spoilage in aquatic food products (Shewan, 1977; Makarios-Laham and Lee 1993; Rodríguez and others 2003a). Asakawa and others (1998) characterized a protease from a *Bacillus* sp. strain isolated from horse mackerel, and which was thought to play a major role in post-mortem decomposition of skin

1 **Figure 2 – Comparative evolution of proteolytic bacteria in horse mackerel muscle**
2 **during storage in either slurry ice (□) or flake ice (◇).**

tissue, this leading to flesh spoilage. Microbial proteolysis of muscle has also been reported to cause sensory spoilage in horse mackerel homogenates stored at 10°C (Kobatake and others 1992). In our work, the evolution of the counts of microorganisms potentially involved in the proteolytic breakdown of horse mackerel was investigated in both batches. As displayed in Figure 2, statistically significant ($p<0.05$) lower counts of proteolytic bacteria were determined in the muscle of horse mackerel kept in slurry ice, as compared with flake ice. The average difference in the counts between batches was 1.43 log units on day 8, and this difference even increased to 2.48 log units after 12 d of storage (Figure 2). The numbers of proteolytic bacteria in the muscle of horse mackerel stored in flake ice reached levels above 10^6 CFU/g by day 12, while the slurry ice batch only reached counts of 10^4 CFU/g or lower, even on day 22, this clearly indicating a significant decrease in the growth of this bacterial group in horse mackerel muscle stored in slurry ice. The results presented here confirm other previous studies indicating a significantly slower growth of proteolytic bacteria in turbot (Rodríguez and others 2003b), and hake (Rodríguez and others 2003c) stored in slurry ice, as compared with flake ice.

The medium-fat nature of horse mackerel makes this fish species especially sensitive to mechanisms involved in lipid damage (Aubourg and Ugliano 2002). Among these mechanisms, the production of extracellular proteases by certain microorganisms may play a role in the lipolytic breakdown of fish species such as albacore tuna (Ben-Gigirey and others, 2000). Accordingly, the evolution of the numbers of microorganisms exhibiting lipolytic activity was investigated in both batches, the results being presented in Figure 3. Statistically significant ($p<0.05$) lower numbers of lipolytic bacteria were determined in the slurry ice batch than in the flake ice batch. On day 8, the average difference in the numbers between both batches was found to be 1.98, this difference increasing up to 4.40 log units after 12 d of storage (Figure 3). The numbers

1 **Figure 3 – Comparative evolution of lipolytic bacteria in horse mackerel muscle**
2 **during storage in either slurry ice (□) or flake ice (◇).**

1 of lipolytic bacteria in the muscle of horse mackerel stored in flake ice reached levels
2 above 10^6 CFU/g by day 12, while the slurry ice batch only reached counts of 10^4
3 CFU/g after 15 d of storage. According to these results, the growth of bacteria
4 potentially involved in the lipolytic breakdown of horse mackerel was slowed down as a
5 consequence of storage in slurry ice. This is, to our knowledge, the first results
6 describing the effects of slurry ice on microbial lipolytic activity in a medium-fat fish
7 species such as horse mackerel.

8 The average counts of anaerobes in muscle of horse mackerel stored in slurry ice
9 were 2.5 log CFU/g, no statistically significant at the $p < 0.05$ level being determined
10 between both batches. The evolution of anaerobes in each batch throughout storage
11 neither evidenced significant differences with respect to the initial counts at day 0 (2.16
12 log CFU/g). These results confirm the notably good initial quality of the fish specimens
13 and the limited growth of anaerobes during chilled storage, regardless of the ice system
14 employed. With respect to the development of coliforms, similar results were obtained.
15 Thus, the average numbers of coliforms throughout storage were very low (< 1 log
16 CFU/g), no statistically significant ($p < 0.05$) difference being observed between both
17 batches, and neither with respect to the initial counts at day 0. Similar low numbers of
18 anaerobes and coliforms had been obtained by Figueroa and others (1990) in jack
19 mackerel.

20 It should also be remarked that the counts of total aerobes, proteolytic and lipolytic
21 bacteria, correlated well with the differences observed in the sensory evaluation, a result
22 that also agreed with previous reports for shrimp (Huidobro and others 2002), turbot
23 (Rodríguez and others 2003b), and hake (Rodríguez and others 2003c) kept in slurry
24 ice. The slowing down effect of storage in slurry ice on the growth of proteolytic and
25 lipolytic bacteria, according to the results presented here, would imply a lower presence
26 of microbial proteases and lipases in the muscle of horse mackerel, this limiting the

1 **Figure 4 – Comparative evolution of pH value in horse mackerel muscle during**
2 **storage in either slurry ice (□) or flake ice (◇).**

negative effects of such resistant enzymes on the lipid compounds of this fish species, since proteases and lipases may retain activity for long periods even at low temperatures (Alford and Pierce 1961).

Chemical analyses

Statistically significant ($p < 0.05$) differences were observed for the pH value in horse mackerel stored in slurry ice and flake ice (Figure 4). Thus, while only slight increases in pH (from the initial 6.38 to a peak of 6.59) were observed in the slurry ice batch, significant pH increases (up to a pH value of 7.65) were determined in the flake ice batch along storage. The remarkable increase of pH in the flake ice batch might indicate a more intense growth of alkalinizing bacteria in such batch, this leading to a higher accumulation of ammonia compounds, with the subsequent negative effects on sensory quality, especially external and flesh odour..

The different evolution of the pH value observed in each batch correlated well with the evolution of TVB-N and TMA-N (Figures 5 and 6, respectively). Thus, the formation of both TVB-N and TMA-N in horse mackerel muscle was slowed down in the slurry ice batch, especially after 8 d and 5 d of storage, respectively. Thus, The TVB-N content (Figure 5) of horse mackerel stored in slurry ice was very low, reaching levels of 31 mg/100 g after 22 days of storage. By contrast, the TVB-N content exhibited a dramatic increase after 8 d of storage in the muscle of horse mackerel stored in flake ice, reaching concentrations as high as 84 mg/100 g after 19 days of storage. Statistical analysis confirmed that the storage of horse mackerel in slurry ice implied a significantly ($p < 0.05$) lower formation of TVB-N as compared with flake ice. Other authors have also reported TVB-N contents higher than 50 mg/100 g in horse mackerel stored for 9 d at 4°C (Kuda and others 2002), this also being far above those determined in this study in horse mackerel stored in slurry ice.

Mejor separa figures 5A y 5B en Figures 5 y 6.

1 **Figure 5 – Determination of (A) total volatile base-nitrogen (TVB-N) and (B)**
 2 **trimethylamine-nitrogen (TMA-N) contents in horse mackerel muscle during**
 3 **storage in either slurry ice (□) or flake ice (◇).**

Likewise, the TMA-N content in muscle also increased very slowly in the period between 0-22 days of storage in the slurry ice batch. After day 5, a sharp increase of the TMA-N content was determined only in the muscle of horse mackerel stored in flake ice. Finally, average TMA-N values as different as 10.5 and 22.5 mg/100 g were determined in the slurry ice batch and flake ice batch, respectively, after 19 days of storage (Figure 5B). As expected from the results obtained in the present study, storage in slurry ice significantly ($p<0.05$) slowed down the formation of TMA-N, especially after 5 d of storage, in comparison with storage in flake ice. Since TMA-N has been reported to the best chemical parameter to determine quality loss on horse mackerel (Aubourg 2001), the benefits of slurry ice for extending the shelf life of horse mackerel should be remarked.

Identification of bacteria potentially involved in the proteolytic and lipolytic breakdown of horse mackerel muscle

There is little previous information available about the identification of spoilage microorganisms from horse mackerel. Silva and others (1998) studied effects of inoculation of five spoilage species into horse mackerel stored under and ozone atmosphere, but several of the microbial species considered were typical spoilage bacteria, not specific microorganisms isolated from horse mackerel. Thus, a qualitative analysis of the predominant proteolytic and lipolytic bacterial strains isolated from horse mackerel muscle stored in liquid ice during 22 d and then subjected to abusive temperatures (30°C) for 3 d was carried out in this work.

From the 26 initial microbial isolates exhibiting proteolytic or lipolytic activity in plate bioassays, 13 isolates showing different phenotypes in the preliminary microbiological study and relative abundance of the total proteolytic or lipolytic colonies in each fish specimen were selected for further study (Table 3). Except for

Table 3 – Identification of microorganisms involved in the proteolytic and lipolytic breakdown of horse mackerel muscle.

Strain	Storage conditions	Proteolytic (P)/ Lipolytic (L) activity ^a	Bacterial species
P1	Slurry ice + 30°C ^b	P (++)/L (++)	<i>Staphylococcus xylosus</i>
P7	Slurry ice + 30°C ^b	P (++)/L (–)	<i>Staphylococcus xylosus</i>
P8	Slurry ice + 30°C ^b	P (+)/L (++)	<i>Proteus penneri</i>
P9	Slurry ice + 30°C ^b	P (++)/L (+)	<i>Proteus penneri</i>
P13	Slurry ice + 30°C ^b	P (++)/L (++)	<i>Staphylococcus xylosus</i>
L1	Slurry ice + 30°C ^b	P (+++++)/L (+)	<i>Proteus penneri</i>
L2	Slurry ice + 30°C ^b	P (+++++)/L (++)	<i>Staphylococcus xylosus</i>
L5	Slurry ice + 30°C ^b	P (+++)/L (+++)	<i>Proteus penneri</i>
L7	Slurry ice + 30°C ^b	P (+++)/L (+++)	<i>Proteus penneri</i>
L8	Slurry ice + 30°C ^b	P (+++)/L (+++)	<i>Proteus penneri</i>
L9	Slurry ice + 30°C ^b	P (+++)/L (+++)	<i>Staphylococcus xylosus</i>
L12	Slurry ice + 30°C ^b	P (+++++)/L (++)	<i>Staphylococcus xylosus</i>
L13	Slurry ice + 30°C ^b	P (++++)/L (++)	<i>Proteus vulgaris</i>

^a Proteolytic/lipolytic activity: + = very weak; ++ = weak; +++ = moderate; ++++ = strong; +++++ = very strong.

^b Isolates were obtained from muscle stored in slurry ice for 22 d and then subjected to abusive temperature conditions for 3 d.

strain P7, that only exhibited a proteolytic phenotype, all the remaining 12 isolates exhibited both proteolytic and lipolytic activities. Six isolates were identified as *Staphylococcus xylosus*, while other six belonged to the species *Proteus penneri* –an indol-negative variant of *Proteus vulgaris*– the other isolate being identified as *Proteus vulgaris* (Table 3). While *Proteus* spp. have been previously isolated from a number of aquatic food products, *S. xylosus* strains have deserved attention of fish technologists because of its ability to biosynthesize and secrete extracellular histidine decarboxylase, this leading to the formation of histamine in seafood products such as semi-preserved anchovies (Rodríguez-Jerez and others 1994).

The phenotypic differences among the proteolytic and lipolytic isolates belonging to the same species were also investigated by the API ZYM system. The results obtained for *S. xylosus* isolates indicated that all the six strains could be classified in three groups: (i) a trypsin-producing, weak proteolytic/weak lipolytic group (isolates P1 and P13), (ii) a trypsin non-producing, strong proteolytic/weak lipolytic group (isolates L2, L9 and L12), and (iii) a trypsin non-producing, moderate proteolytic/non-lipolytic strain (isolate P7). Accordingly, it was concluded that at least three different *S. xylosus* strains, these were P1, L2 and P7, belonged to the microflora of horse mackerel, the other *S. xylosus* strains being multiple isolates of any of such three strains. Interestingly, all *S. xylosus* strains isolated from horse mackerel biosynthesized and secreted alkaline phosphatase, acid phosphatase, leucine aryl amidase, naphthol-phosphohydrolase and α -glycosidase.

With respect to the *P. penneri* strains, two of them –P8 and P9– produced cysteine aryl amidase, showed a weak production of extracellular lipases and proteases and almost equivalent phenotypic profiles. Isolates L5, L7 and L8 of *P. penneri* did not produce cysteine aryl amidase and exhibited moderate proteolytic/moderate lipolytic activity. Finally, isolate L1 did not produce cysteine aryl amidase and exhibited very

1 strong proteolytic/weak lipolytic activity. Thus, at least three different *P. penneri* strains
2 –P8, L5 and L1– were isolated from horse mackerel under abusive temperature
3 conditions. Interestingly, all *P. penneri* strains isolated from horse mackerel also
4 biosynthethized and secreted alkaline phosphatase, acid phosphatase, leucine aryl
5 amidase, naphtol-phosphohydrolase and α -glycosidase.

6 Thus, the results obtained in this work suggest that *P. penneri* and *S. xylosus* are
7 involved in the proteolytic and lipolytic breakdown of horse mackerel muscle. In
8 addition to these two species, a moderately lipolytic/strong proteolytic strain of *Proteus*
9 *vulgaris* was also isolated. The fact that these three species produce glycosidic enzymes
10 can enhance their proteolytic and lipolytic activity. Thus, such enzymes, together with
11 their bacterial proteases and lipases, can degrade cell membranes and expose proteins
12 and lipids to the respective action of proteases and lipases (Marin and Marshall 1983;
13 Marin and others 1984). The fact that slurry ice significantly slowed down the growth of
14 proteolytic and lipolytic bacteria in the muscle of horse mackerel, as described above,
15 underlines the benefits that such storage system may exert on the maintenance of quality
16 and shelf life of this fish species.

CONCLUSIONS

In summary, from the results of the sensory analyses presented here, storage of horse mackerel in slurry ice allows a better maintenance of quality and enhances the shelf life of this fish species more than 10 d: from 5 d (flake ice batch) to 15 d (slurry ice batch). Storage in slurry ice was accompanied by significantly lower counts of total aerobes, proteolytic and lipolytic bacteria, better control of pH and a slowed down formation of both TMA-N and TVB-N. The good results obtained in the microbiological, sensory, and chemical analyses strongly suggest the use of slurry ice to improve the chilled commercialization of horse mackerel.

REFERENCES

- Alford JA, Pierce DA. 1961. Lipolytic activity of microorganisms at low and intermediate temperatures. III. Activity of microbial lipases at temperatures below 0°C. *J Food Sci* 26:518.
- Antonacopoulos N. 1960. Verbesserte apparatus zur quantitativer destillation wasserdampf-flüchtiger stoffe. *Z Lebensm Unters Forsch* 13:113–160.
- Asakawa M, Sadakata Y, Araki T, Sumi T, Nakagawa H. 1998. Purification and characterization of the alkaline serine protease produced by *Bacillus* sp. N4 strain from fish skin mucus. *Fish Sci* 64:793–797.
- Aubourg S. 2001. Damage detection in horse mackerel (*Trachurus trachurus*) during chilled storage. *J Am Oil Chem Soc* 78:857–862.
- Aubourg S, Sotelo C, Gallardo J. 1997. Quality assessment of sardines during storage by measurement of fluorescent compounds. *J Food Sci* 62:295–299.
- Aubourg SP, Ugliano M. 2002. Effect of brine pre-treatment on lipid stability of frozen horse mackerel (*Trachurus trachurus*). *Eur Food Res Technol* 215:91–95. Mejor eliminar ya que es de congelado y no tiene análisis de TVB ni TMA.
- Bandarra NM, Batista I, Nunes ML, Empis JM. 2001. Seasonal variation in the chemical composition of horse mackerel (*Trachurus trachurus*). *Eur Food Res Technol* 212:535–539.
- Ben-Gigirey B, Vieites Baptista de Sousa JM, Villa TG, Barros-Velázquez J. 1998. Changes in biogenic amines and microbiological analysis in albacore (*Thunnus alalunga*) muscle during frozen storage. *J Food Prot* 61:608–615.
- Ben-Gigirey B, Vieites Baptista de Sousa JM, Villa TG, Barros-Velázquez J. 1999. Histamine and cadaverine production by bacteria isolated from fresh and frozen albacore (*Thunnus alalunga*). *J Food Prot* 62:933–939.

- 1 Ben-Gigirey B, Vieites-Baptista de Sousa JM, Villa TG, Barros-Velázquez J. 2000.
2 Characterization of biogenic amine-producing *Stenotrophomonas maltophilia* strains
3 isolated from white muscle of fresh and frozen albacore tuna. Int J Food Microbiol
4 57:19–31.
- 5 Chapman L. 1990. Making the grade. Ice slurries get top marks for quality products.
6 Austral Fish 7:16–19.
- 7 Chinivasagam HN, Bremner HA, Wood AF, Nottingham SM. 1998. Volatile
8 components associated with bacterial spoilage of tropical prawns. Int J Food
9 Microbiol 42:45–55.
- 10 DOCE. 1989. Baremo de clasificación de frescura. In: Diario Oficial de las
11 Comunidades Europeas. Brussels: European Comission. p. 5–6.
- 12 FAO Inform. 1998. Fishery statistics. In: Food and Agriculture Organization of The
13 United Nations Yearbook 1996. Rome, Italy: FAO. p 187–188.
- 14 Figueroa G, Galeno H, Troncoso M, Aguilera JM. 1990. Análisis of the microbial flora
15 of jack mackerel (*Trachurus murphyi*) minced products. Sci Alim 10:907–912.
- 16 García I, Pérez-Villarreal B, Pozo R. 1996. Processing of underutilized fish species.
17 Aliment Equip Tecnol 15:145–149.
- 18 Gram L, Huss H. 1996. Microbiological spoilage of fish and fish products. Int J Food
19 Microbiol 33:121–137.
- 20 Harada K. 1991. How to handle albacore. Austral Fish 2:28–30.
- 21 Hsieh R, Kinsella J. 1989. Oxidation of polyunsaturated fatty acids: mechanisms,
22 products, and inhibition with emphasis on fish. Adv Food Res Nutr Res 33:233–341.
- 23 Huidobro A, Mendes R, Nunes ML. 2001. Slaughtering of gilthead seabream (*Sparus*
24 *aurata*) in liquid ice: influence on fish quality. Eur Food Res Technol 213:267–272.

- 1 Huidobro A, López-Caballero M, Mendes R. 2002. Onboard processing of deepwater
2 pink shrimp (*Parapenaeus longirostris*) with liquid ice: Effect on quality. Eur Food
3 Res Technol 214:469–475.
- 4 Kobatake M, Kreger van Rij, NJW, Placido MTLC, Uden N van. 1992. Isolation of
5 proteolytic psychrotrophic yeasts from fresh raw seafoods. Lett Appl Microbiol
6 14:37–42.
- 7 Kraus L. 1992. Refrigerated sea water treatment of herring and mackerel for human
8 consumption. In: Burt J, Hardy R, Whittle K, editors. Pelagic Fish. The Resource and
9 its Exploitation. Aberdeen, Scotland: Fishing News Books. p. 73–81.
- 10 Kuda T, Matsumoto C, Yano T. 2002. Changes in acid and alkaline phosphatase
11 activities during the spoilage of raw muscle from horse mackerel (*Trachurus*
12 *japonicus*) and gurnard (*Lepidotrigla microptera*). Food Chem 76:443–447.
- 13 Makarios-Laham IK, Lee TC. 1993. Protein hydrolysis and quality deterioration of
14 refrigerated and frozen seafood due to obligately psychrophilic bacteria. J Food Sci
15 58:310–313.
- 16 Marin A, Marshall RT. 1983. Production of glycosidases by psychrotrophic bacteria. J
17 Food Sci 48:570.
- 18 Marin A, Marshall RT. 1984. Glycosidic activities of *Pseudomonas fluorescens* on fat-
19 extracted skim milk, buttermilk, and milk fat globule membranes. J Dairy Sci 67:52.
- 20 Merck Microbiology Manual. 2002. Coliforms and *E. coli*. VRB Agar.
21 (<http://service.merck.de/microbiology>).
- 22 Monteagudo Torres S, Montaña Miguelez J, Míguez Bernárdez M. 2002. Comparison
23 of sensory and physicochemical methods for evaluating the quality and commercial
24 life of lean fish (*Trisopterus luscus*) and fatty fish (*Trachurus trachurus*) marketed in
25 Pontevedra. Alimentaria 334:73–79.

- 1 Nunes M, Batista I, Morão de Campos R. 1992. Physical, chemical and sensory analysis
2 of sardine (*Sardina pilchardus*) stored in ice. J Sci Food Agric 59:37–43.
- 3 Phaff HJ, Starmer WT, Lachance MA, Ganter PF. 1994. *Candida caseinolytica* sp. nov.,
4 a new species of yeast occurring in necrotic tissues of *Opuntia* and *Stenocereus*
5 species in the Southwestern United States and Baja California, Mexico. Appl
6 Environ Microbiol 44:641–645.
- 7 Pigott G, Tucker B. 1987. Science opens new horizons for marine lipids in human
8 nutrition. Food Rev Int 3:105–138.
- 9 Price RJ, Melvin EF, Bell JW. 1991. Postmortem changes in chilled round, bled and
10 dressed albacore. J Food Sci 56:318–321.
- 11 Rodríguez O, Barros-Velázquez J, Ojea A, Piñeiro C, Aubourg S. 2003a. Evaluation of
12 sensory and microbiological changes and identification of proteolytic bacteria during
13 the iced storage of farmed turbot (*Psetta maxima*). J Food Sci 68(9–10):in press.
- 14 Rodríguez O, Barros-Velázquez J, Ojea A, Piñeiro C, Gallardo JM, Aubourg S. 2003b.
15 Effect of chilled storage in flow ice on the microbial quality and shelf life of farmed
16 turbot (*Psetta maxima*). Isolation and identification of major proteolytic bacteria. In:
17 Proceedings of the First Joint Trans-Atlantic Fisheries Technology Conference,
18 TAFT 2003, 33rd WEFTA and 48th AFTC Meetings. Reykjavik, Iceland. p. 73–74.
- 19 Rodríguez O, Losada V, Aubourg S, Barros-Velázquez J. 2003c. Enhanced shelf-life of
20 chilled European hake (*Merluccius merluccius*) stored in slurry ice as determined by
21 sensory analysis and assessment of microbiological activity. Food Res Int
22 (submitted).
- 23 Rodríguez-Jerez JJ, Mora-Ventura MT, López-Sabater EI, Hernández-Herrero M. 1994.
24 Histidine, lysine, and ornithine decarboxylase bacteria in Spanish salted semi-
25 preserved anchovies. J Food Prot 57:784–7, 791.

- 1 Shewan JM. 1977. The bacteriology of fresh and spoiling fish and the biochemical
2 changes induced by bacterial action. In: Handling, Processing and Marketing of
3 Tropical Fish. London: Tropical Product Institute. p. 51–68.
- 4 Silva MV da, Gibbs PA, Kirby RM. 1998. Sensorial and microbial effects of gaseous
5 ozone on fresh scad (*Trachurus trachurus*). J Appl Microbiol 84:802–810.
- 6 Simeonidou S, Govaris A, Vareltzis K. 1997. Quality assessment of seven
7 Mediterranean fish species during storage in ice. Food Res Int 30:479–484.
- 8 Statsoft. 1994. Statistica for Macintosh. Tulsa, OK: Statsoft and its licensors.
- 9 Tabara Y, Ueki S, Ito M, Watanabe T, Kan T, Hiraoka Y, Nakajima S, Tsuchiya T.
10 1998. [FALTA TITULO](#). J Jap Soc Food Sci Technol 45:93–99.
- 11 Tozawa H, Erokibara K, Amano K. 1971. Proposed modification of Dyer's method for
12 trimethylamine determination in cod fish. In: Kreuzer R, editor. Fish Inspection and
13 Quality Control. London: Fishing News Books. p. 187–190.
- 14 Whittle K, Hardy R, Hobbs G. 1990. Chilled fish and fishery products. In: Gormley T,
15 editor. Chilled Foods: the State of the Art. New York: Elsevier Applied Science. p.
16 87–116.

1 **ACKNOWLEDGMENT**

2 The authors wish to thank KINARCA S.A.U. for providing the slurry ice equipment.

3 This work was supported by two projects granted by the Secretaría Xeral de I+D from
4 the Xunta de Galicia (Project PGIDT01MAR40202PR and Project
5 PGIDTI02RMA18E). The authors also thank Ms. Marta Torres, Mr. Marcos Trigo and
6 Mr. José M. Antonio for their excellent technical assistance.

7