EFFECT OF SLURRY ICE ON CHEMICAL CHANGES
RELATED TO QUALITY LOSS DURING EUROPEAN HAKE
(*Merluccius merluccius*) CHILLED STORAGE

Vanesa Losada¹, Carmen Piñeiro¹, Jorge Barros-Velázquez² and Santiago
P. Aubourg^{1,*}

¹Department of Seafood Chemistry; Institute for Marine Research (CSIC), C/ Eduardo
Cabello 6, 36208-Vigo (Galicia, Spain)

²Department of Analytical Chemistry, Nutrition and Food Science, School of Veterinary
Sciences, University of Santiago de Compostela, 27002-Lugo (Galicia, Spain)

*Correspondent: Fax: +34 986 292762; e-mail: saubourg@iim.csic.es

ABSTRACT

Slurry ice is a biphasic system consisting of small spherical ice crystals surrounded by seawater at subzero temperature. Its employment was evaluated in the present work as a new chilled storage method for whole European hake (*Merluccius merluccius*) and compared to traditional flake icing. Different chemical analyses (nucleotide degradation, formaldehyde formation, lipid hydrolysis and oxidation, interaction compounds formation and electrophoretic protein profiles) related to quality changes were analysed and compared to sensory evaluation. An inhibitory effect on quality loss mechanisms was observed for the slurry ice treatment, according to nucleotide degradation (K value), free formaldehyde content, browning development and sarcoplasmic protein profiles. No differences in lipid hydrolysis and oxidation could be outlined by comparison between both icing conditions. The sensory analysis showed a higher shelf-life time for slurry icing than flake icing (12 days and 5 days, respectively). Results confirm the practical advantages of using slurry ice as a chilling storage method.

Keywords: Chilled storage, slurry ice, flake ice, hake, chemical changes, sensory assessment, quality, shelf-life

Running title: Slurry ice and hake quality

INTRODUCTION

Marine species deteriorate rapidly post-mortem. This degradation process is carried out at first stage by muscle enzymes and later by microbial enzymes. The rate of alteration depends on factors such as the nature of the fish species, size, lipid content, biological status at the moment of capture, importance and nature of the microbial load, and storage temperature [1-3]. With a view to slow down the mechanisms involved in quality loss, the fish should be refrigerated immediately after capture. Thus, different preservative methods such as traditional flake ice [4], refrigerated seawater [5] and addition of chemical preservation agents [6, 7] have been applied to fish species.

Recently, slurry ice, also known as fluid ice, slush ice, liquid ice, flow ice or binary ice, has been reported to be a promising technique for the preservation of aquatic food products in an ice-water suspension at subzero temperature [8-10]. Two relevant characteristics of slurry ice are a) its faster chilling rate, which is a consequence of its higher heat-exchange capacity, and b) the reduced physical damage caused to food products by its microscopic spherical particles as compared with the damage elicited by flake ice. In addition, complete coverage of the fish surface by the slurry ice mixture affords a better protection of the fish material with respect to oxidation and dehydration events, while its versatility may combine it with other agents such as ozone and melanosis inhibitors.

Although the theoretical advantages of slurry ice are well known, few empirical data reporting the potential practical advantages derived from the use of slurry ice for the storage of marine species are available. For fish species, good results were obtained with slurry ice for the on-board storage of albacore tuna (*Thunnus alalunga*) [11], but no significant spoilage differences were obtained between flake and slurry ices in the

case of a warm-water fish species such as sea bass (*Dicentrarchus labrax*) [12]. When applied to seabream (*Sparus aurata*), slurry ice proved to be a good slaughter method to sacrifice marine species leading to beneficial storage conditions [13]. For crustacean species, practical advantages were obtained in the case of Australian prawns [14] and shrimp (*Parapenaeus longirostris*) [10].

In Spain, hake (*Merluccius merluccius*) is a fish species of a great market demand [15] because of its excellent organoleptic features. The capture of European hake in distant fishing banks usually means that the time elapsed between the catch and arrival to destination may vary from five to fifteen days, this underlining the need to optimise on-board refrigeration parameters in order to provide consumers with fish of the highest quality degree possible. In the present work, the effect of slurry ice on European hake was studied by comparison with the traditional flake ice treatment. Chemical analyses related to quality loss were checked and compared to sensory evaluation.

MATERIALS AND METHODS

Slurry ice system and traditional flake ice

A slurry ice prototype (FLO-ICE, Kinarca S.A.U., Vigo, Spain) was employed. 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 $-1.5\text{ }^{\circ}\text{C}$. Flake ice was prepared with an Icematic F100 Compact device (CASTELMAC SPA, Castelfranco, Italy); the temperature of the flake ice was $-0.5\text{ }^{\circ}\text{C}$.

The fish specimens were surrounded by slurry or flake ice at a 1:1 fish to ice ratio, and stored in a refrigerated room at 2°C. When required, flake ice and the slurry ice mixture were renewed.

Fish material, processing and sampling

European hake (*Merluccius merluccius*) specimens were caught (February, 2003) near the Galician Atlantic coast and transported on ice to the laboratory six hours after catching. The fish specimens were not headed nor gutted and were directly placed in slurry ice or flake ice. The length of the specimens was in the range of 25-30 cm and the weight was 150-200 g. Three different groups were used for each icing treatment and studied separately along the whole experimental period. Samples were taken for analysis on days 0, 2, 5, 8, 12, 15, 19, and in the case of slurry ice, also at day 22.

Once fish specimens had been subjected to sensory analyses, the white muscle was separated and used for chemical analyses. All analyses were performed in triplicate.

Composition analyses

Water content was determined by the difference between the weight of fresh homogenized muscle (1-2 g) and the weight recorded after 24 h at 105 °C. Results were expressed as g water/100 g muscle. Lipids were extracted by the Bligh and Dyer [16] method. Quantification results were expressed as g lipid /100 g wet muscle.

Chemical changes related to quality loss

Free fatty acid (FFA) content was determined by the Lowry and Tinsley [17] method based on complex formation with cupric acetate-pyridine. Results were expressed as g FFA/100 g lipids.

The peroxide value (PV), expressed as meq oxygen /kg lipid, was determined by the ferric thiocyanate method [18].

Formation of fluorescent compounds were determined with a Perkin Elmer LS 3B fluorimeter by measurements at 393/463 nm and 327/415 nm, as previously described [19, 20]. The relative fluorescence (RF) was calculated as follows: $RF = F/F_{st}$, where F is the fluorescence measured at each excitation/emission maximum, and F_{st} is the fluorescence intensity of a quinine sulphate solution (1 µg/mL in 0.05 M H₂SO₄) at the corresponding wavelength. The fluorescence ratio (FR) was calculated as the ratio between the two RF values: $FR = RF_{393/463 \text{ nm}}/RF_{327/415 \text{ nm}}$. The FR value was determined in the aqueous phase resulting from the lipid extraction [16].

Browning development was measured from the lipid extract at 450 nm and 400 nm. The 450/400 absorbance ratio (browning ratio, BR) was studied according to Hassan et al. [21].

Nucleotide analysis was carried out according to Ryder [22]. The K value was calculated according to the following concentration ratio: $K \text{ value} = 100 \times (\text{hypoxanthine} + \text{inosine}) / (\text{adenosine triphosphate} + \text{adenosine diphosphate} + \text{adenosine monophosphate} + \text{inosine monophosphate} + \text{inosine} + \text{hypoxanthine})$.

Extraction of free formaldehyde (FA) from fish muscle was carried out according to Rey-Mansilla et al. [23]. FA content was determined by the spectrophotometric (Beckman Coulter DU 640 spectrophotometer) method of Nash [24] and expressed as µg FA/g fish muscle.

Changes in sarcoplasmic protein profiles were detected by means of SDS-PAGE analyses in commercial Excel-Gel SDS (Amersham Biosciences, Sweden) according to Piñeiro et al. [25]. Sarcoplasmic protein extracts were prepared in a low-ionic-strength buffer composed of 10 mM Tris-HCl, pH 7.2 + 50 mM PMSF (pentamethyl sulphonic

acid). Portions of 500 mg of muscle were homogenized for 60 s in 4 mL of the buffer solution. Then, extracts were centrifuged at 12,500 rpm for 15 min. in a JA20.1 rotor (J221-M centrifuge, Beckman-Coulter, London, UK) at 4°C, and the supernatants were recovered. All extracts were maintained at –80°C until analysis. Protein concentrations in the extracts were determined by means of the protein microassay method (Bio-Rad Hercules, CA, USA). A standard curve constructed for bovine serum albumin was used as reference. The mobility of protein bands was determined using the PDQUEST Software (Bio-Rad, Hercules, CA, USA) and the corresponding molecular weights were determined by comparison with the size of the protein standards. Gel staining was carried out by silver staining.

Sensory analyses

Sensory analysis was conducted by a sensory panel consisting of five experienced judges, according to traditional guidelines concerning fresh and refrigerated fish [26, 27]. Four categories were ranked: highest quality (E), good quality (A), fair quality (B), and unacceptable quality (C). Sensory assessment of the fish included the following parameters: skin (colour, slime), external odour, gills (colour, odour), consistency and flesh odour.

Statistical analyses

Biochemical data corresponding to both chilling methods were subjected to one-way analysis of variance to assess significant ($p < 0.05$) differences between both treatments [28]. The SPSS 11.5 software for windows (SPSS Inc., Chicago, IL, USA) was also used to explore the statistical significance of the results obtained, this

including multivariate contrasts and multiple comparisons by the Scheffé and Tuckey tests; a confidence interval at the 95% level ($p < 0.05$) was considered in all cases.

RESULTS AND DISCUSSION

Water contents of the different fish samples were in the range 78 – 83 %, while the lipid content ranged between 0.30 and 0.50 %. Both results agreed to previous hake studies [20, 29]. Differences in both constituents may be explained as a result of fish-to-fish variations and not derived from chilling conditions and storage time.

Nucleotide analyses

Nucleotide autolytic degradation was studied by means of the K value. Its assessment in both icing conditions (Table 1) showed significantly higher values ($p < 0.05$) for the flake ice treatment. In this sense, a day-to-day comparison showed higher values at days 8, 12, 15 and 19 for the batch stored in traditional flake ice. Accordingly, it is concluded that the application of slurry ice slows down the nucleotide degradation pathway in hake. Along the storage time, both treatments showed gradual increases of the K value with time, this leading to K values close to 25 and 54 at day 19, in the slurry ice batch and flake ice batch, respectively.

Formaldehyde formation

Hake is a well known FA producer as a result of autolytic trimethylamine oxide (TMAO) breakdown [30, 31] during storage conditions. In the present case, formaldehyde was produced during both icing conditions (Table 1). However, an inhibitory effect of slurry ice treatment was concluded on the autolytic TMAO

breakdown, since its employment lead to a lower free formaldehyde formation. From day 5 and onwards, higher mean values were obtained for the flake ice treatment, this being significantly higher than the slurry ice counterparts at days 5, 12 and 19. Production of FA was negligible till day 2 (flake ice) and day 5 (slurry ice); then, a gradual increase was observed in both batches reaching at day 19 values about 6 and 20 for slurry and flake ices, respectively.

Lipid damage

The FFA formation was quite similar between batches and, therefore, did not provide a significant difference ($p < 0.05$) between both icing treatments (Table 2). Accordingly, an inhibitory effect of slurry ice on lipid hydrolysis development could not be concluded. Thus, FFA formation exhibited a slow and gradual increase along the storage time in both batches. Mean values obtained at the end time (2.14 and 2.51 for slurry and flake ices, respectively) were relatively low as compared to fatty (sardine) [19] and medium fat (horse mackerel) [32] fish species stored in flake conditions.

Lipid oxidation was followed by the peroxide assessment (Table 2). Mean values obtained did not provide clear tendencies with the time of storage in both treatments. A day-to-day comparison between both icing conditions showed some differences in the 5-12 days period. However, if the whole process is considered, an inhibitory effect of slurry ice could not be concluded. Values obtained showed to be relatively low ($PV < 6.0$ in all cases) when compared to other fish species [33, 34].

Interaction compounds formation

Interaction compounds formation between protein-like compounds and electrophilic substances (aldehydes and peroxides, namely) produced during the chilled

storage was studied by means of the fluorescence ratio (Table 3). Its assessment did not show a different ($p < 0.05$) behaviour between both treatments. However, at day 19 a higher level was obtained for the ice batch. This fluorescence development is difficult to be explained in terms of lipid oxidation compounds formation, according to the low peroxide development. However, according to previous research [35, 36], FA presence has shown to enhance the fluorescent compounds formation. In the present case, a higher FA production in flake ice could be responsible for a higher FR value at the end of the storage.

Interaction compounds formation was also studied by means of the browning compound formation (Table 3). In this case a significantly lower ($p < 0.05$) browning development could be assessed for the slurry ice treatment. Samples stored under traditional ice showed higher mean values than their counterparts along the whole experiment. An inhibitory effect of slurry ice on interaction compound formation is concluded for this kind of detection.

Electrophoretic profiles of sarcoplasmic proteins

Previous reports by other authors have proposed certain soluble polypeptides as spoilage or freshness biomarkers [37]. Such polypeptides would be produced as a result of proteolytic processes happened in the myofibrillar and sarcoplasmic fractions [38, 39]. These observations agree with the results obtained in this work. Thus, and as it can be observed in Figure 1, the profiles obtained from the electrophoretic analyses of sarcoplasmic proteins show differences between batches as the time of storage progressed. In this way, a correlation between the spoilage degree and the appearance of two polypeptides (about 23 and 24.5 kDa) could be observed. The amount of such polypeptides increased between days 5-12 of storage in the specimens stored in flake

ice, while such polypeptides were detected in the slurry ice batch only after 12 days of storage. Currently, the usefulness of the referred polypeptides as quality biomarkers of quality in hake muscle is being investigated at our laboratory.

Sensory analyses

Hake specimens stored in slurry ice maintained good quality (category A) up to day 8 (Table 4). After this time, sensory quality decreased and on day 15 this batch was no longer acceptable for marketing. The main negative aspect related to quality loss in the slurry ice group corresponded to the appearance of the eyes. In a previous work related to seabream (*Sparus aurata*) [13], eyes appearance was also reported to be the limiting factor. Parameters that deserved the best scores were the gills and the flesh odour, which were judged to keep acceptable rates of decomposition even at day 22 of storage.

On contrast, specimens stored in traditional flake ice (Table 4), maintained good quality (category A) only until day 2. Afterwards, sensory quality decreased and the batch exhibited unacceptable quality on day 8. In this batch, the limiting factor was the gill odour and colour.

Previous research on chilled European hake [29, 40] has accounted for longer shelf-life times (20-25 days). In our work, the shelf-life of the hake batches was not long. This difference should be explained because of the relatively small size of the hake specimens employed in our study (150-200g), while in the above-mentioned reports [29, 40], hake specimens exhibited a larger size (325-500g and 245-430g, respectively). In the present study, the use of hake specimens of small size was considered so that differences between both chilling treatments could be easily outlined.

FINAL COMMENTS

The storage of European hake in slurry ice has shown an inhibitory effect on relevant chemical mechanisms (nucleotide degradation, formaldehyde production, interaction compound formation and electrophoretic sarcoplasmic protein profiles) related to quality loss. These results correlated well with the respective shelf-lives determined by sensory assessment. On contrast, no significant effect of slurry ice could be detected on lipid hydrolysis and oxidation. These results confirm the practical advantages of using slurry ice as a chilling storage method for a lean fish species of relevant commercial value, such as European hake, and encourage its employment on all kinds of marine species.

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FIGURE LEGENDS

Figure 1: Electrophoretic profiles of the sarcoplasmic proteins of hake muscle by means of SDS-PAGE. Different lines include a standard (st; bovine serum albumin) product and the different storage times (days) for both icing conditions. Black arrows highlight the accumulation of polypeptides of 23 kDa and 24.5 kDa as storage progressed.

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TABLE 1

K value and free formaldehyde (FA) assessment during chilled (slurry and flake icing)
hake storage*

Storage Time (days)	K Value (%)		Free Formaldehyde (µg/g fish muscle)	
	Slurry Ice	Flake Ice	Slurry Ice	Flake Ice
2	5.88 ± 2.66	11.12 ± 4.45	0.04 ± 0.01	0.04 ± 0.01
5	8.55 ± 2.50	12.85 ± 3.86	0.04 ± 0.01 a	1.37 ± 0.31 b
8	12.05 ± 1.51 a	16.63 ± 1.14 b	1.13 ± 0.14	1.16 ± 0.59
12	14.63 ± 6.05 a	34.53 ± 8.01 b	1.33 ± 0.73 a	9.82 ± 0.79 b
15	18.51 ± 4.54 a	30.94 ± 9.38 b	4.61 ± 2.76	7.48 ± 2.77
19	25.16 ± 4.06 a	54.36 ± 4.93 b	6.27 ± 1.05 a	20.36 ± 3.44 b
22	20.24 ± 3.31		6.45 ± 0.17	

*Mean value ± standard deviation is indicated. For each index, values followed by different letters indicate significant differences (p<0.05) between both icing treatments. Raw fish values: 4.77 ± 0.63 (K value) and 0.04 ± 0.01 (FA).

TABLE 2

Lipid damage assessment during chilled (slurry and flake icing) hake storage*

Storage Time (days)	Free Fatty Acids (FFA) (g/100g lipid)		Peroxide Value (PV) (meq oxygen/kg lipid)	
	Slurry Ice	Flake Ice	Slurry Ice	Flake Ice
2	1.09 ± 0.09	1.07 ± 0.21	0.69 ± 0.16	1.67 ± 0.08
5	0.82 ± 0.27	1.11 ± 0.34	5.35 ± 0.94 b	2.55 ± 0.60 a
8	1.17 ± 0.32	1.25 ± 0.02	2.84 ± 0.08 a	3.70 ± 0.38 b
12	1.54 ± 0.28	1.37 ± 0.22	2.68 ± 1.55 b	2.14 ± 1.01 a
15	1.57 ± 0.20	1.61 ± 0.16	2.60 ± 0.08	4.03 ± 2.73
19	2.14 ± 0.18	2.51 ± 0.42	2.48 ± 0.37	2.04 ± 0.54
22	2.28 ± 0.35		2.31 ± 1.03	

*Mean value ± standard deviation is indicated. For each index, values followed by different letters indicate significant differences ($p < 0.05$) between both icing treatments. Raw fish values: 0.62 ± 0.08 (FFA) and 0.63 ± 0.30 (PV).

TABLE 3

Interaction compound assessment* during chilled (slurry and flake icing) hake
storage**

Storage Time (days)	Fluorescence Ratio (FR)		Browning Ratio (BR)	
	Slurry Ice	Flake Ice	Slurry Ice	Flake Ice
2	0.57 ± 0.07	0.51 ± 0.05	0.48 ± 0.11	0.52 ± 0.04
5	0.47 ± 0.05	0.64 ± 0.06	0.32 ± 0.16	0.50 ± 0.10
8	0.55 ± 0.04	0.57 ± 0.04	0.54 ± 0.16	0.62 ± 0.13
12	1.32 ± 0.67	1.03 ± 0.42	0.50 ± 0.01 a	0.67 ± 0.04 b
15	1.59 ± 0.92	1.86 ± 0.42	0.54 ± 0.04	0.63 ± 0.05
19	1.09 ± 0.25 a	2.01 ± 0.53 b	0.54 ± 0.07	0.70 ± 0.18
22	0.83 ± 0.17		0.52 ± 0.03	

*Both ratios were measured according to the Materials and Methods section.

**Mean value ± standard deviation is indicated. For each index, values followed by different letters indicate significant differences ($p < 0.05$) between both icing treatments. Raw fish values: 0.68 ± 0.14 (FR) and 0.45 ± 0.04 (BR).

TABLE 4

Sensory assessment during chilled (slurry and flake icing) hake storage*

Icing	Storage Time (days)						
Conditions	2	5	8	12	15	19	22
Slurry Ice	A	A	A	B	C	C	C
Flake Ice	A	B	C	C	C	C	

*Freshness categories: E (excellent), A (good), B (fair) and C (unacceptable). Raw fish was category E.