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Food Chemistry 93 (2005) 619–625
Food Chemistry
www.elsevier.com/locate/foodchem

# Inhibition of chemical changes related to freshness loss during storage of horse mackerel (*Trachurus trachurus*) in slurry ice

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Received 2 August 2004; received in revised form 28 September 2004; accepted 28 September 2004

**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 horse mackerel (*Trachurus trachurus*) and compared with traditional flake icing. Different chemical analyses (nucleotide degradation, lipid hydrolysis and oxidation, interaction compounds formation and electrophoretic protein profiles) related to quality loss were checked and compared to sensory evaluation. An inhibitory effect on quality loss mechanisms was observed for the slurry ice treatment, according to the assessment of the *K* value, free fatty acid content, thiobarbituric acid index, fluorescent compounds formation and sarcoplasmic protein profiles. The sensory analysis showed a higher shelf-life time for fish treated under slurry icing than for flake iced fish (15 and 5 days, respectively). Results confirm the practical advantages of using slurry ice as a chilling storage method. According to the inhibition of lipid hydrolysis and oxidation obtained, the employment of slurry ice on relatively fat fish species is recommended to obtain safer and higher quality fish products.
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*Keywords:* Slurry ice; Flake ice; Horse mackerel; Damage pathways; Chemical changes; Sensory assessment; Quality; Shelf- life

## 1. Introduction

During chilled storage of fish, significant deterioration of sensory quality and loss of nutritional value have been detected as a result of changes in chemical constituents, that lead to a strong effect on the commercial value (Ashie, Smith, & Simpson, 1996; Olafsdóttir et al., 1997; Whittle, Hardy, & Hobbs, 1990). This degradation process is carried out in the initial 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, state at the moment of capture, importance and nature of the microbial load, and storage temperature. With the aim of reducing loss in freshness, different preservative methods, such as traditional flake ice (Nunes, Batista, & Morâo de Campos, 1992), refrigerated sea water (Kraus, 1992) and chemical addition (Hwang & Regenstein, 1995; Ponce de León, Inoue, & Shinano, 1993) have been employed.

Slurry ice, also known as fluid ice, slush ice or liquid ice, has been reported to be a promising technique for the preservation of aquatic food products in an ice–water suspension at subzero temperature (Chapman, 1990; Harada, 1991). Slurry ice has been shown to provide several advantages over flake ice, such as: lower temperature, faster chilling, lower physical damage to product and better heat exchange power.

Although the theoretical advantages of using 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.

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doi:10.1016/j.foodchem.2004.09.041

Thus, good results were obtained with slurry ice for the on-board storage of albacore tuna (Price, Melvin, & Bell, 1991), but no significant spoilage differences were obtained between flake and slurry ice when applied to a warm-water fish species (sea bass, *Dicentrarchus labrax*) (Martinsdóttir, Valdimarsdóttir, Porkelsdóttir, Olafsdóttir, & Tryggvadóttir, 2002). For seabream (*Sparus aurata*), holding in slurry ice was shown to be a good method of sacrificing marine species and it also leads to beneficial stored conditions (Huidobro, Mendes, & Nunes, 2001). For crustacean species, practical advantages were obtained in the case of Australian prawns (Chinivasagam, Bremner, Wood, & Nottingham, 1998) and shrimp (Huidobro, López-Caballero, & Mendes, 2002).

Horse mackerel (*Trachurus trachurus*) is an underutilised medium-fat content fish, abundant in the Northeast Atlantic (FAO, 2003). It has recently attracted great attention because of its moderate price and large quantities captured by most West-European countries (Holland, Ireland, Spain, France, Germany and Portugal). Efforts have been made to utilize them as smoked (García, 1996), canned (Münkner, 1987), chilled (Aubourg, 2001), frozen (Simeonidou, Govaris, & Vareltzis, 1997) and restructured (Alvarez-Parrilla, Puig, & Lluch, 1997) fish products.

In the present work, the effect of slurry ice on horse mackerel was studied by comparison with the traditional flake ice treatment. Chemical analyses related to different damage pathways and quality losses were checked and compared by sensory evaluation.

## 2. Materials and methods

### 2.1. 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 °C. Flake ice was prepared with an Icematic F100 Compact device (CASTELMAC SPA, Castelfranco, Italy); the temperature of the flake ice was −0.5 °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, the flake ice and the slurry ice mixture were renewed.

### 2.2. Fish material, processing and sampling

Fresh horse mackerel (*Trachurus trachurus*) specimens were caught near the Galician Atlantic coast (November, 2002) and transported in ice to the laboratory 10 h after catching. The fish specimens were not headed nor gutted and were directly placed in slurry ice or flake ice in an isothermal room at 2 °C. The length of the specimens was in the range of 25–30 cm and the weight was 200–250 g. Three different groups were used for each icing treatment and were studied separately throughout 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 employed for chemical analyses. All analyses were performed in triplicate.

### 2.3. Composition analyses

Water content was determined as 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 $kg^{-1}$ muscle. Lipids were extracted by the Bligh & Dyer (1959) method. Quantification results were expressed as g lipid $kg^{-1}$ wet muscle.

NaCl content in fish muscle was calculated from the amount of chloride by boiling in $HNO_3$ with excess of $AgNO_3$, followed by titration with $NH_4SCN$ (AOAC, 1990). Results were expressed as g NaCl $kg^{-1}$ muscle.

### 2.4. Chemical changes related to quality loss

Nucleotide analysis was carried out according to Ryder (1985). The *K* value was calculated according to the following concentration ratio: *K* value = 100 × (hypoxanthine + inosine)/(adenosine triphosphate + adenosine diphosphate + adenosine monophosphate + inosine monophosphate + inosine + hypoxanthine).

Free fatty acid (FFA) content was determined by the Lowry & Tinsley (1976) method, based on complex formation with cupric acetate–pyridine. Results were expressed as g FFA $kg^{-1}$ lipids.

The thiobarbituric acid index (TBA-i) was determined according to Vyncke (1970). Results were expressed as mg malondialdehyde $kg^{-1}$ fish sample.

Formation of fluorescent compounds was determined with a Perkin–Elmer LS 3B fluorimeter by measurements at 393/463 nm and 327/415 nm, as previously described (Aubourg, Medina, & Gallardo, 1998; Aubourg, Sotelo, & Gallardo, 1997). 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 $ml^{-1}$in 0.05 M $H_2SO_4$) at the corresponding wavelength. The fluorescence ratio (FR) was calculated as the ratio between the two RF values: $FR = RF_{393/463\ nm}/RF_{327/415\ nm}$. The FR value was determined in the aqueous phase resulting from the lipid extraction (Bligh & Dyer, 1959).

Changes in sarcoplasmic protein profiles were detected by means of SDS–PAGE analyses in commercial

Excel–Gel SDS (Amersham Biosciences; Uppsala, Sweden) according to Piñeiro et al. (1999). Sarcoplasmic protein extracts were prepared in a low-ionic-strength buffer composed of 10 mM Tris–HCl, pH 7.2 + 50 mM pentamethyl sulfonic acid (PMSF). 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 with bovine serum albumin 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 positions of the protein standards. Gel staining was carried out by silver staining according to manufacture protocol.

### 2.5. Sensory analysis

Sensory analysis was conducted by a sensory panel consisting of five experienced judges, according to guidelines concerning fresh and refrigerated fish (Council Regulation, 1990). 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, external odour, gills, consistency and flesh odour.

### 2.6. 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 treatments (Statsoft, 1994). 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.

## 3. Results and discussion

### 3.1. Composition analyses

Water contents of the different fish samples were in the range 770–810 g kg$^{-1}$, while the lipid contents ranged between 12 and 37 g kg$^{-1}$. Both results agreed with previous horse mackerel studies (Aubourg, 2001; Aubourg, Lehmann, & Gallardo, 2002). Differences in both constituents may be attributed to fish-to-fish variation and not to chilling conditions or storage time.

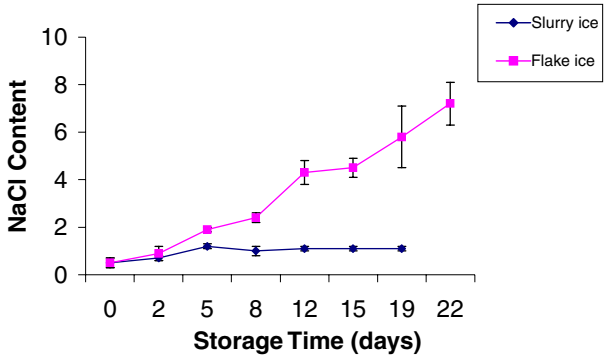


Fig. 1. Comparative evolution of NaCl content in horse mackerel during storage in slurry ice and in flake ice (g NaCl kg$^{-1}$). Bars denote standard deviation of the mean ($n = 3$).

The presence of NaCl in the chilling medium led, in the slurry ice treatment, to a progressive increase of NaCl content in fish white muscle (Fig. 1) reaching, at the end of the experiment, 7.2 ± 0.9 g kg$^{-1}$. This NaCl content increase is found to be much smaller than that obtained after refrigeration in seawater (Smith, Hardy, McDonald, & Templeton, 1980), salting (Srikar, Khuntia, Reddy, & Srinivasa, 1993; Thorarinsdottir, Arason, Geirsdottir, Bogason, & Kristbergsson, 2002) or salting, followed by smoking (Jittinandana, Kenney, Slider, & Kiser, 2002).

### 3.2. Nucleotide analysis

Nucleotide autolytic degradation was studied by means of the *K* value (Fig. 2). Both icing conditions showed increases during storage, according to previous research on horse mackerel chilled under traditional conditions (Smith, McGill, Thomson, & Hardy, 1980). In the present work, flake ice treatment showed significantly higher ($p < 0.05$) values than did slurry ice. Differences between both treatments increased with time so that, at days 12, 15 and 17, the *K* values for samples

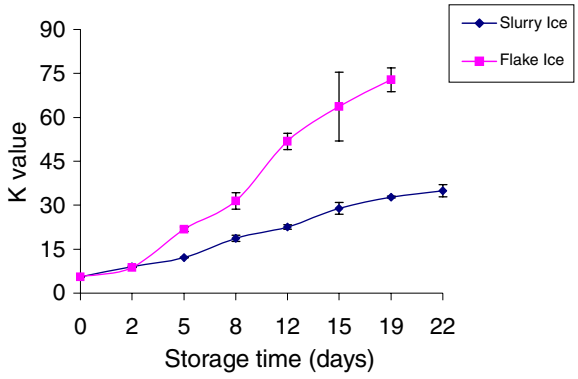


Fig. 2. Comparative evolution of *K* value in horse mackerel during storage in slurry ice and in flake ice. Bars denote standard deviation of the mean ($n = 3$).

treated under flake ice were more than double those of samples chilled under slurry ice conditions. It is concluded that the application of slurry ice slows down the nucleotide degradation pathway in horse mackerel.

### 3.3. Lipid damage

Lipid hydrolysis was determined according to the FFA determination (Fig. 3). FFA content of the raw material (3.90 ± 0.90) was rather similar to those of fatty fish species (tuna, sardine) (Aubourg et al., 1997; Gallardo, Aubourg, & Pérez-Martín, 1989) and lower than those of lean fish (blue whiting, haddock, cod) (Aubourg & Medina, 1999; Aubourg et al., 1998). In the present experiment, up to day 12 of storage, the FFA content evolution was quite similar under both conditions of icing, showing a slight increase with time. Then, a lower FFA formation was observed for fish samples treated under slurry ice than in their counterparts stored under flake ice conditions. Accordingly, an inhibitory effect on lipid hydrolysis could be concluded.

The formation of FFA itself does not lead to nutritional losses. However, its examination was deemed to be important since it has been proved that the accumulation of FFA is related to some extent to texture deterioration by interacting with proteins (Mackie, 1993; Sikorski & Kolakowska, 1994) and to lipid oxidation enhancement (Miyashita & Takagi, 1986; Yoshida, Kondo, & Kajimoto, 1992).

Secondary oxidation was followed by the TBA-i (Fig. 4). Its assessment in both icing conditions showed a general increase for all treatments. When compared with the starting fish material, a significant increase ($p < 0.05$) was obtained at day 5 in both cases. Comparison between the two icing conditions showed higher values ($p < 0.05$) for the flake ice treatment. A day-to-day comparison showed higher values at days 5, 15 and 19 for the batch stored in traditional flake ice. It is concluded that the application of slurry ice slows down the formation of thiobarbituric acid reactive substances in horse mackerel.

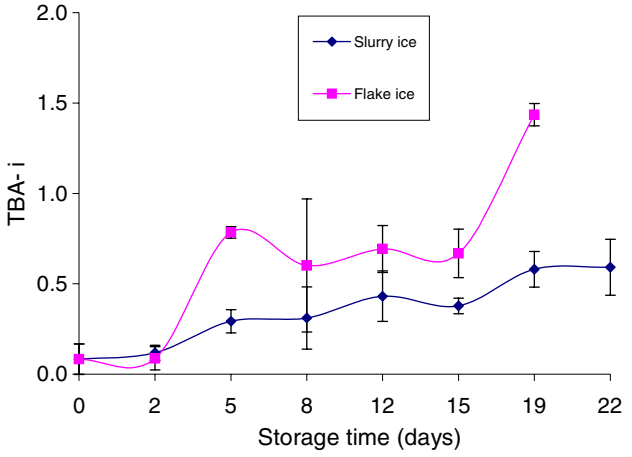


Fig. 4. Comparative evolution of thiobarbituric acid index (TBA-i) in horse mackerel during storage in slurry ice and in flake ice. Bars denote standard deviation of the mean ($n = 3$).

### 3.4. Interaction compounds analysis

Interaction compound formation (Gardner, 1979; Leake & Karel, 1985) between protein-like compounds and electrophilic substances (aldehydes and peroxides) produced during the chilled storage was studied by means of the fluorescence ratio (Aubourg et al., 1997, 1998). Up to day 8, its assessment (Fig. 5) did not show different ($p < 0.05$) behaviour between the two treatments. Then, a sharp increase was observed for fish samples that were treated under flake ice. In the case of the slurry ice treatment, a significant increase of the fluorescence ratio was not observed throughout the entire

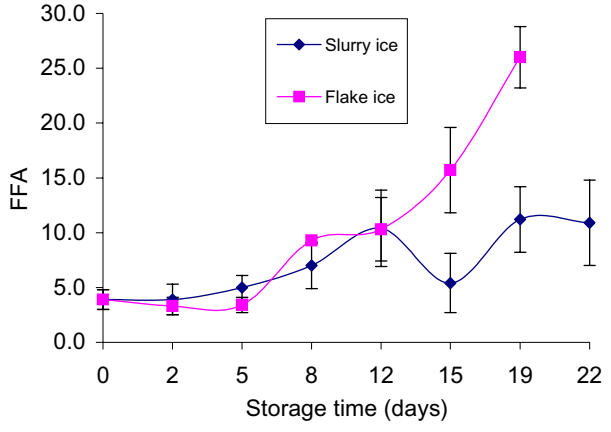


Fig. 3. Comparative evolution of free fatty acid (FFA) content in horse mackerel during storage in slurry ice and in flake ice (g FFA $kg^{-1}$). Bars denote standard deviation of the mean ($n = 3$).

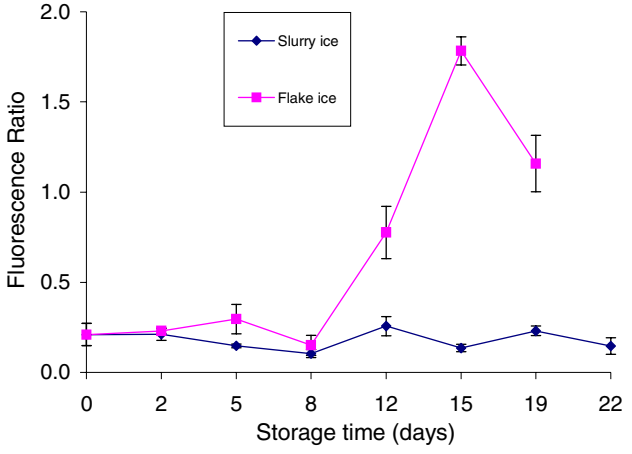


Fig. 5. Comparative evolution of fluorescent compounds (fluorescence ratio) formation in horse mackerel during storage in slurry ice and in flake ice. Bars denote standard deviation of the mean ($n = 3$).

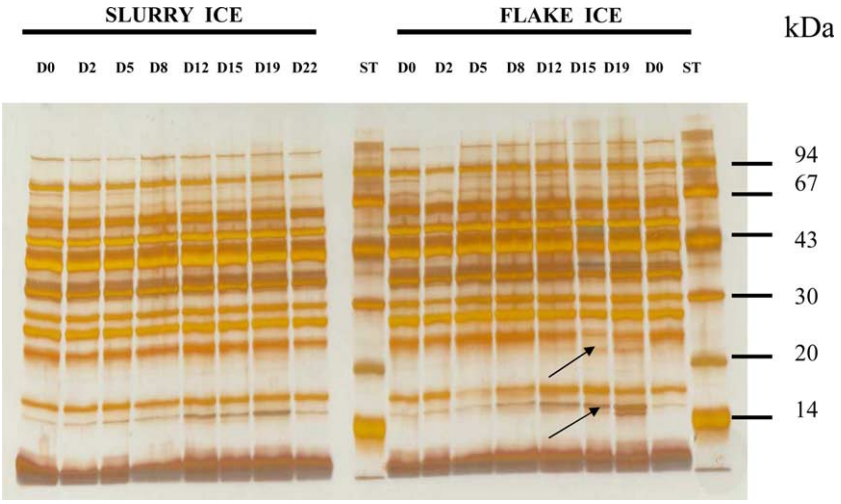


Fig. 6. Comparative electrophoretic profiles obtained in 15% homogeneous SDS–ExcelGel from sarcoplasmic proteins of horse mackerel white muscle during storage in slurry ice and in flake ice. Different lanes include a low molecular weight standard (ST; 14–94 kDa) and the different storage times (D0–D22, from day 0 till day 22). Arrows indicate the new bands.

experiment, which agrees with the relatively low thiobarbituric acid reactive substance formation (Fig. 4). An inhibitory effect on interaction compounds formation is concluded for the slurry ice employment.

### 3.5. Electrophoretic profiles of sarcoplasmic proteins

Previous reports by other authors have proposed certain soluble polypeptides as spoilage or freshness biomarkers (Morzel, Vérrez-Bagnis, Arendt, & Fleurence, 2000). Such polypeptides would be produced as a result of proteolytic processes affecting the myofibrillar and sarcoplasmic fractions (Papa, Alvarez, Vérrez-Bagnis, Fleurence, & Benyamin, 1996; Vérrez-Bagnis, Noel, Sautereau, & Fleurence, 1999). These observations agree with the results obtained in this work. Thus, and as can be observed in Fig. 6, the profiles obtained from the electrophoretic analyses of sarcoplasmic proteins show differences between batches as the time of storage progresses. In this way, a correlation between the degree of spoilage and the appearance of two polypeptides (25 and 14 kDa) could be observed. The amount of such polypeptides increased at day 19 for the specimens stored in flake ice, while such polypeptides could not be detected in the slurry ice batch throughout the entire experiment.

### 3.6. Sensory analysis

Horse mackerel specimens stored in slurry ice maintained good quality (categories E and A) up to day 8 (Table 1). After this time, sensory quality decreased and on day 19 this batch was no longer acceptable. Parameters that showed the worst scores were the eyes, gills and flesh odour, whereas the best score was the consistency, which was judged to keep acceptable rates of decomposition even at day 22 of storage. In a previous work related to slurry ice treatment of seabream (*Sparus aurata*) (Huidobro et al., 2001), the appearance of the eyes was also reported to be the limiting factor.

Table 1
Sensory assessment during chilled (slurry and flake icing) horse mackerel storage[a]

| Icing conditions | Storage time (days) | | | | | | |
|---|---|---|---|---|---|---|---|
| | 2 | 5 | 8 | 12 | 15 | 19 | 22 |
| Slurry ice | E | A | A | B | B | C | C |
| Flake ice | E | B | C | C | C | C | |

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

By contrast, specimens stored in flake ice (Table 1), maintained good quality (categories E and A) only until day 2. After this time, sensory quality decreased and the batch exhibited unacceptable quality on day 8. In this batch, the limiting factors were the gills and the flesh odour. An important improvement of sensory parameters is achieved by employing the slurry ice treatment.

## 4. Conclusions

The storage of horse mackerel specimens in slurry ice conditions allowed an inhibitory effect on chemical changes related to quality loss. Thus, development of different fish damage pathways, such as nucleotide autolytic degradation, lipid hydrolysis and oxidation, non-enzymatic browning and protein profile modifications, showed a partial inhibition. These results agreed with the sensory assessment, which showed a marked increase in shelf life and good quality period when the slurry ice treatment was employed.

An increase of NaCl content in the white muscle of horse mackerel treated under slurry ice was detected. However, values obtained remained relatively low when compared to those observed for salting processes and sea water refrigeration. NaCl content attained at day 15 (shelf life time in the present experiment) was $4.5 \pm 0.4$ g kg$^{-1}$.

In previous research, slurry ice has been tested as a preserving method during on-board cooling and as a slaughtering method for preserving flesh quality. Thus, different quality parameters have been evaluated, such as sensory analysis, physical properties, microbiological activity, nucleotide degradation and volatile amine formation (Huidobro et al., 2001, 2002; Martinsdóttir et al., 2002; Morzel, Sohier, & Van de Vis, 2002). However, the effect of slurry ice on lipid damage has not been previously studied.

Marine lipids are known to be constituted of highly unsaturated fatty acids (Ackman, 1989) that are known to be very prone to oxidation (Harris & Tall, 1994; Kolakowska, 2002), leading to an important effect on fish quality loss (Madrid, Madrid, & Madrid, 1994; Pigott & Tucker, 1987). In this sense, the present work provides the first study where in slurry ice treatment has shown an inhibitory effect on both hydrolytic and oxidative damage. Its application to relatively fat fish species is recommended since its employment would guarantee a longer shelf life, and a fish product showing a smaller freshness loss would be well received by the consumer.

The effect of previous chilling conditions (storage time, fish–ice ratio, room storage temperature) on the quality of frozen (Aubourg et al., 2002; Undeland & Lingnert, 1999) and canned (Aubourg & Medina, 1997; Slabyj & True, 1978) fish has been demonstrated. In this sense, the application of slurry ice technology could lead to higher quality fish products, according to the inhibition of chemical change pathways showed in this manuscript.

## Acknowledgements

The authors thank KINARCA S.A.U. for providing the slurry ice equipment. This work was supported by the Secretaría Xeral de I + D from the Xunta de Galicia (Galicia, Spain) through the research project PGIDIT02RMA18E. The authors also thank Mr. Marcos Trigo, Mr. José M. Antonio and Mrs. Lorena Barros for their excellent technical assistance.

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