

1 Effect of previous chilled storage on rancidity development in frozen horse mackerel (*Trachurus*
2 *trachurus*)

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7 **RANCIDITY DEVELOPMENT IN FROZEN**
8 **PELAGIC FISH: INFLUENCE OF SLURRY ICE AS**
9 **PRELIMINARY CHILLING TREATMENT**

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ABSTRACT

The slurry ice technology has shown profitable advantages when employed instead of traditional flake ice for the manufacture of chilled aquatic species. The present work is aimed at evaluating the effect of slurry ice as a preliminary treatment prior to frozen storage. For it, specimens of a small pelagic fatty fish species (sardine; *Sardina pilchardus*) were stored in slurry ice for 2, 5 and 9 days, then subjected to freezing (-80°C ; 24 h) and finally kept frozen (-20°C) during 1, 2 and 4 months. At such times, rancidity development in frozen sardine was measured by sensory (odour, skin, colour and flesh appearance) and biochemical (lipid hydrolysis and oxidation) analyses and compared to a control batch previously chilled in flake ice. Sensory analysis indicated an extended shelf-life time for frozen sardine that was preliminary stored under slurry ice for 2, 5 or 9 days, as compared to their counterparts subjected to flake icing. Sensory results were corroborated ($p<0.05$) by biochemical lipid oxidation indices (thiobarbituric acid reactive substances and the fluorescence formation). The present work opens the way to the use of slurry ice instead of flake ice as preliminary treatment of fish material prior to the frozen storage.

Running Title: Rancidity in frozen fish and preliminary slurry ice chilling

Keywords: Sardine, slurry ice, frozen storage, rancidity, shelf-life

1. INTRODUCTION

The freezing and frozen storage of fish have been largely used to retain their sensory and nutritional properties before consumption (Madrid, Madrid & Madrid, 1994; Erickson, 1997). Nevertheless, the presence of highly unsaturated lipids in fish and its high content in prooxidant molecules may lead to the development of important enzymatic and nonenzymatic rancidity mechanisms that may limit the shelf-life of fish during its frozen storage, especially in the case of fatty fish species (Hsieh & Kinsella, 1989; Richards & Hultin, 2002).

Before the freezing step is accomplished, adequate storage techniques of fish material should be employed to reduce post-capture losses. Thus, previous research has shown a strong influence of the preliminary chilling time on frozen fish quality (Deng, 1978; Sankar & Viswanathan Nair, 1988; Undeland & Lingnert, 1999; Aubourg, Lehmann & Gallardo, 2002). Among the different handling systems that efficiently cool fish, the most commonly employed is traditional flake icing (Whittle, Hardy & Hobbs, 1990; Nunes, Batista & Morão de Campos, 1992), although other preservation strategies, such as the use of refrigerated sea water (Smith, Hardy, McDonald & Templeton, 1980; Kraus, 1992) or chemical additives (Ponce de León, Inoue & Shinano, 1993; Hwang & Regenstein, 1995) have also been reported.

The introduction of chilling systems based on slurry ice –also known as fluid ice, slush ice, flow ice or liquid ice– has afforded several advantages as compared to traditional flake icing: (i) the sub-zero storage temperature of slurry ice, (ii) its faster chilling rates due to the higher heat exchange power, (iii) the limited physical damage caused to the fish surface due to its microscopic spherical crystals, and (iv) the prevention of dehydration events due to the full coverage of the fish surface (Chapman,

1 1990; Harada, 1991; Piñeiro, Barros-Velázquez & Aubourg, 2004). In this sense, recent
2 studies have reported significant inhibitory effects of slurry ice on the microbiological
3 and biochemical mechanisms involved in fish spoilage, as compared to traditional flake
4 icing. Such studies have thus described important increases in the shelf-lives of a broad
5 variety of chilled aquatic food products such as lean fish (Losada, Piñeiro, Barros-
6 Velázquez & Aubourg, 2004a), medium-fat fish (Losada, Piñeiro, Barros-Velázquez &
7 Aubourg, 2005), fatty fish (Price, Melvin & Bell, 1991; Losada, Barros-Velázquez,
8 Gallardo & Aubourg, 2004b) and crustaceans (Chinivasagam, Bremner, Wood &
9 Nottingham, 1998; Huidobro, López-Caballero & Mendes, 2002).

10 Small pelagic fatty fish species constitute food products of great economic
11 importance in many European countries (FAO, 2005a). Although they are recognised as
12 healthy foods, their short shelf-life during frozen storage strongly limits the
13 commercialisation. One abundant such species in West-European countries and in
14 Morocco is Atlantic sardine (*Sardina pilchardus*), a fish species belonging to the
15 *Clupeidae* family that exhibits an increasing commercial importance and consumer
16 demand (FAO, 2005b). The present study is aimed at improving the commercialisation
17 of frozen sardine by the application of a preliminary chilling step in slurry ice. Thus, the
18 main goal of this work is to explore the potential benefits of slurry icing as a
19 preliminary treatment prior to the freezing and frozen storage, with respect to traditional
20 flake icing. The effects of both icing conditions on the rancidity development of the
21 frozen fish, on its sensory quality and shelf-life are discussed below.

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2. MATERIALS AND METHODS

2.1. Refrigeration systems

A slurry ice prototype (FLO-ICE, Kinarca S.A.U., Vigo, Spain) was used. The composition of the slurry ice binary mixture was 40% ice/60% water, prepared from filtered seawater (salinity: 3.3 ‰). The temperature of the slurry ice mixture was -1.5°C . The average temperature of the specimens processed in slurry ice was in the range of -1.0°C to -1.5°C .

Flake ice was prepared with an Icematic F100 Compact device (Castelmac Spa, Castelfranco, Italy). The temperature of the flake ice was $+0.5^{\circ}\text{C}$. The average temperature of the specimens stored in flake ice was in the range of $+0.5$ to $+1.0^{\circ}\text{C}$.

The fish specimens were surrounded by slurry or flake ice at a 1:1 fish to ice ratio, and stored in an isothermal refrigerated room at $+2^{\circ}\text{C}$. When required, the flake ice and the slurry ice mixture were renewed.

2.2. Raw fish, sampling and processing

Sardine (*Sardina pilchardus*) specimens were caught near the Galician Atlantic coast (North-western Spain) in November 2003 and transported on ice to the laboratory during the first 10 h after the capture. The length of the specimens was in the 16-21 cm range and the average weight of the specimens was 150 g.

Upon arrival in the laboratory the fish specimens were neither headed nor gutted and placed in slurry ice or flake ice before being stored in the isothermal room at $+2^{\circ}\text{C}$. Three different groups of three fishes were considered for each icing treatment and studied separately throughout the whole experimental period to achieve the statistical study. Fish specimens were taken and packaged in individually celled polyethylene bags

1 for the freezing process after 2, 5 and 9 days of chilled storage under each icing
2 conditions. Freezing was carried out at -80°C for 24 h. After that time, all fish
3 specimens were stored under -20°C . Analysis of frozen fish samples was undertaken
4 after 1, 2 and 4 months of storage at -20°C .

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6 **2.3. Sensory analysis**

7 Sensory analysis was conducted by a taste panel consisting of five experienced
8 judges in fish sensory quality assessment, according to the guidelines presented in Table
9 1 (Council Regulations, 1989). Four categories were ranked: highest quality (E), good
10 quality (A), fair quality (B) and rejectable quality (C). Sensory assessment included the
11 evaluation of the following parameters: external odour, external appearance (skin and
12 colour) and flesh appearance. Rancid odour and yellowish colour were chosen as being
13 directly related to the rancidity development. Sour odour and pigmentation losses were
14 also determined as indicators of potential autolytic and microbial breakdown
15 mechanisms during the preliminary chilled storage. Flesh appearance (dryness,
16 myotome degradation and the development of yellowish colour) was considered as an
17 indicator of muscle modifications during processing.

18 At each sampling time, fish specimens from each batch were thawed and
19 analysed in the same session. The panel members shared samples tested. The fish were
20 served to the panel members inside the individual polyethylene bags where they had
21 been kept frozen. Once the fish were subjected to sensory analysis, the white muscle
22 was separated and homogenised to perform all biochemical analyses.

23

1 **2.4. Composition analysis**

2 Water content was determined by weight difference between the homogenised
3 fish muscle (1-2 g) and after 24 h at 105°C. Results were calculated as g water/100 g
4 fish muscle.

5 The lipid fraction was extracted from the fish muscle by the Bligh and Dyer
6 (1959) method. Quantification results were expressed as g lipid/100 g fish muscle.

7 NaCl content in fish muscle was calculated from the amount of chlorine
8 extracted by boiling in the presence of HNO₃. This was followed by the addition of
9 AgNO₃ whose excess was determined by titration with NH₄SCN (AOAC, 1990).
10 Results were expressed as g NaCl/100 g fish muscle.

11

12 **2.5. Lipid damage measurements**

13 Free fatty acids (FFA) content was determined according to Lowry and Tinsley
14 (1976), with the only modification of replacing benzene by toluene. This method is
15 based on a complex formation between the acid group of FFA and cupric acetate in the
16 presence of pyridine at pH = 6.1; the resulting chromophore is determined by
17 absorbance measurement at 710 nm. Results were expressed as g oleic acid/ 100 g
18 lipids.

19 The peroxide value (PV) was determined according to the Chapman and McKay
20 (1949) method. Results were expressed as meq active oxygen/kg lipids. Briefly, the
21 ferric ions, formed by the oxidation of ferrous ions by peroxides, react with thiocyanate
22 leading to the development of a pink-purple colour that can be measured by absorbance
23 reading at 500 nm.

24 The thiobarbituric acid index (TBA-i) (mg malondialdehyde/kg fish muscle) was
25 determined according to Vyncke (1970). This method is based on the reaction between a

1 trichloroacetic acid extract of the fish muscle, and thiobarbituric acid at high temperature
2 (95-97°C), the resulting chromophore being measured at 532 nm.

3 Fluorescent compounds were measured at excitation/emission wavelengths of
4 327/415 nm and 393/463 nm on the lipid extracts as described elsewhere (Aubourg,
5 Sotelo & Pérez-Martín, 1998). Fluorescence measurements (Perkin-Elmer LS 3B) of
6 samples were normalised with quinine sulphate measurements, so that relative
7 fluorescence (RF) was calculated as follows: $RF = F/F_{st}$, where F is the fluorescence
8 measured at each excitation/emission pair, and F_{st} is the fluorescence intensity of a
9 quinine sulphate solution (1 µg/ ml in 0.05 M H₂SO₄) at the corresponding wavelength.
10 Results were expressed as the fluorescence ratio (FR) obtained, according to the
11 following calculation: $FR = RF_{393/463nm} / RF_{327/415nm}$.

12

13 **2.6. Statistical analyses**

14 Data from the different measurements were subjected to the one-way ANOVA
15 method ($p < 0.05$). Comparison of means was performed using a least-squares difference
16 (LSD) method (Statsoft, 1994). Statistical differences between preliminary slurry ice
17 and flake ice storage conditions were analysed. Quality differences in frozen fish as a
18 result of the frozen storage time and the preliminary chilled storage time in each of the
19 chilling conditions were also studied.

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3. RESULTS AND DISCUSSION

3.1. Composition analysis

For all kinds of samples studied under the present experiment, the water content of sardine muscle was in the range of 70.5-73.5 %, while the lipid content ranged between 2.5% and 4.5%. Average starting fresh fish values of 71.8 ± 0.5 and 3.5 ± 0.3 for water and lipid contents, respectively, were determined. Small differences in both constituents were observed among the different kinds of samples, a result that may be explained in terms of fish-to-fish variations rather than derived from the preliminary chilling conditions, chilling time or frozen storage time.

NaCl content in frozen fish muscle that was preliminary stored under flake ice conditions ranged between 0.06% and 0.11% and did not exhibit significant differences ($p>0.05$) as a result of chilling time and frozen storage time. The presence of NaCl in the slurry ice chilling medium led to progressive increases ($p<0.05$) of the NaCl content in frozen fish muscle, depending on the preliminary chilling time (Figure 1). Previous studies have shown that the NaCl content increases in chilled fish muscle after storage in slurry ice (Losada et al., 2004b; Losada et al., 2005). In the present study, NaCl concentrations determined for frozen fish that had been preliminary chilled under slurry ice conditions during 5 and 9 days, resulted to be significantly higher ($p<0.05$) than their counterparts preliminary stored in flake ice. Remarkably, the NaCl content was not affected ($p>0.05$) by the frozen storage time when shorter preliminary chilling times (2 and 5 days) were considered. However, the extension of the chilling time to 9 days conducted to a lower ($p<0.05$) NaCl content in fish muscle kept frozen during 1 month, as compared to their counterparts stored frozen for 2 and 4 months.

1 **3.2. Lipid hydrolysis**

2 The evolution of lipid hydrolysis is presented in Table 2. Preliminary icing
3 conditions produced slight differences. The higher FFA formation was observed in fish
4 subjected to 4 months of frozen storage with preliminary 2 days or 5 days slurry ice
5 treatment, as compared to their counterparts treated under flake ice conditions.
6 However, the opposite result between both icing conditions was obtained when
7 considering fish muscle chilled for 5 days and then kept frozen during 1 month. As a
8 consequence of these results, a definitive effect of the preliminary slurry ice treatment
9 on the lipid hydrolysis development during sardine frozen storage could not be
10 concluded.

11 Different mechanisms involved in lipid hydrolysis can be discussed at the light
12 of the processes considered in this study. On one hand, FFA formation has been
13 described to be inhibited in the presence of NaCl (Refsgaard, Brockhoff & Jensen,
14 2000; Losada et al., 2004b; Losada et al., 2005). In this sense, preliminary storage in
15 slurry ice should limit the rate of lipid hydrolysis events. On the other hand, storage in
16 slurry ice exerts a very limited damage on fish proteins when compared to flake ice
17 conditions (Losada et al., 2004a; Losada et al., 2005), so that the activity of enzymes
18 responsible for lipid hydrolysis (mainly lipases and phospholipases) could be more
19 intense during subsequent frozen storage when a preliminary slurry ice treatment is
20 employed. According to the latter effect, a higher FFA formation would be expected to
21 occur in the batch preliminary stored in slurry ice.

22 In our study, the FFA formation exhibited a progressive increase ($p < 0.05$) along
23 the time of frozen storage in both batches, this being in agreement to previous works
24 describing notable rates of lipid hydrolysis mechanisms in different types of fish during
25 its frozen storage (Aubourg et al., 1998; Refsgaard et al., 2000). However, in our study,

1 the duration of the preliminary chilled storage did not produce significant effects
2 ($p>0.05$) on the FFA formation during subsequent frozen storage, this being in
3 agreement with previous research (Aubourg et al., 2002).

4 While the formation of FFA itself does not lead to nutritional losses, its
5 assessment is deemed important when considering the rancidity development. Thus, a
6 pro-oxidant effect of FFA on lipid matter has been proposed, and explained on the basis
7 of a catalytic effect of the carboxyl group on the formation of free radicals by the
8 decomposition of hydroperoxides (Yoshida, Hondo & Kajimoto, 1992; Aubourg, 2001).
9 Moreover, and being relatively small-size molecules, FFA have shown to undergo a
10 faster oxidation rate than bigger lipid classes (triglycerides and phospholipids, namely)
11 (Labuza, 1971; Miyashita & Takagi, 1986), this significantly affecting the sensory
12 quality of aquatic food products (Refsgaard et al., 2000).

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14 **3.3. Lipid oxidation**

15 Several lipid oxidation indices were assessed to follow up the development of
16 rancidity in frozen sardine. For it, primary (peroxides), secondary (thiobarbituric acid
17 reactive substances, TBARS) and tertiary (interaction compounds) lipid oxidation were
18 investigated.

19 According to the peroxide value, no clear effect of the preliminary chilling
20 treatment on lipid oxidation could be appraised (Table 3). Thus, preliminary storage in
21 slurry ice for 2 or 5 days implied lower ($p<0.05$) PV after 1 month of frozen storage, as
22 compared with the counterpart batches stored under flake ice conditions. However, in
23 the case of fish subjected to 4 months of frozen storage, a preliminary chilling for 5
24 days under slurry ice led to a higher PV as compared to the counterpart batch chilled in
25 flake ice.

1 A progressive peroxide formation ($p < 0.05$) along the frozen storage time was
2 observed in all batches, this indicating a close relationship between the development of
3 rancidity and the time of frozen storage. Preliminary chilling time only exerted a
4 significant effect ($p < 0.05$) on rancidity in the case of fish specimens kept frozen for 1
5 and 2 months (Deng, 1978; Sankar & Viswanathan Nair, 1988; Undeland & Lingnert,
6 1999; Aubourg et al., 2002) while no effect was observed ($p > 0.05$) for specimens
7 subjected to 4 months of frozen storage.

8 The results concerning secondary lipid oxidation are presented in Table 4. As
9 expected, TBARS formation exhibited an increasing ($p < 0.05$) tendency as the
10 preliminary chilling time (Deng, 1978; Aubourg et al., 2002) and frozen storage time
11 (Aubourg et al., 1998) progressed, this indicating a progressive quality loss.
12 Comparison between both kinds of icing conditions showed that the TBARS value of
13 fish specimens kept frozen for 4 months exhibited lower values in specimens
14 preliminary chilled for 5 or 9 days under slurry ice conditions. Similar results were
15 observed in sardine specimens subjected to two months of frozen storage after a
16 preliminary period of 5 days of chilled storage in slurry ice. According to most previous
17 research, present results on TBARS have been expressed as mg malondialdehyde/kg
18 fish muscle; however, when results obtained are expressed on lipid basis (mg
19 malondialdehyde/kg lipids), the same differences are obtained between both preliminary
20 chilling conditions. As a consequence of TBARS results, it can be concluded that
21 preliminary slurry ice chilling step provided a limitation of oxidation development of
22 frozen sardine.

23 Tertiary lipid oxidation events were investigated by measuring the formation of
24 interaction compounds (Pokorný, 1981; Howell, 1995) between primary and secondary
25 lipid oxidation products and nucleophilic molecules (protein-like, namely) present in the

1 fish muscle. The results of the fluorescence ratio (FR) values are presented in Table 5.
2 A slow development of the FR value was obtained in all batches, when compared to
3 previous research conducted on chilled and frozen seafood (Aubourg et al., 1998;
4 Aubourg et al., 2002). In the actual study, a significant difference ($p < 0.05$) was
5 observed when comparing both preliminary icing conditions. Thus, in the case of fish
6 specimens subjected to the longest chilling and frozen storage times (9 days and 4
7 months, respectively), slurry ice treatment has led to limitation of interaction compound
8 formation, this result being in agreement with the above-mentioned results concerning
9 TBA-i and PV.

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11 **3.4. Sensory analysis**

12 Sensory scores obtained by the frozen sardine batches are displayed in Table 6.
13 As expected, a progressive quality loss was observed as a result of increasing the
14 preliminary chilling storage (Sankar & Viswanathan, 1988; Aubourg et al., 2002) and
15 the frozen storage time (Undeland, Ekstrand & Lingnert, 1998; Aubourg et al., 1998;
16 Saeed & Howell, 2002). Sardine specimens that had been preliminary stored in flake ice
17 were found acceptable until month 1 of frozen storage if maintained chilled during 2 or
18 5 days. More extended chilled storage times (9 days) led to unacceptable scores in the
19 frozen fish, even at the lowest time of frozen storage (1 month). If preliminary treated
20 under slurry ice, sardine specimens were found acceptable even after two months of
21 frozen storage at any of the chilling times considered (2, 5 and 9 days). A shelf-life
22 increase in frozen sardine is concluded when slurry ice is employed as preliminary
23 chilling storage instead of the traditional flake icing conditions.

24 The main concern related to the loss of sensory quality in frozen fish previously
25 chilled in slurry ice was the flesh appearance (2 days chilling time), flesh appearance

1 and external odour (5 days chilling time) and all attributes (9 days chilling time). In the
2 case of the preliminary flake ice chilling, the limiting factor in frozen fish resulted to be
3 the flesh appearance (2 days chilling time), flesh appearance, skin and external colour (5
4 days chilling time) and all attributes (9 days chilling time).

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4. FINAL REMARKS

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9 Marine lipids are reported to be constituted of highly unsaturated fatty acids very
10 prone to oxidation from capture and leading to an important effect on fish quality loss
11 (Pigott & Tucker, 1987; Kolakowska, 2002). As a consequence of this, on-board and
12 transport steps have to employ the chilling storage before the definite technology
13 treatment can be applied. Previous research (Losada et al., 2004a; Losada et al., 2004b;
14 Losada et al., 2005) has shown an inhibitory effect of slurry ice on lipid hydrolysis and
15 oxidation events in chilled fish. In the present work, the employment of slurry ice as a
16 preliminary storage system prior to the freezing of sardine was evaluated with the aim
17 of elucidating its potential benefits on the control of rancidity development in the frozen
18 product. In all cases, the preliminary slurry ice chilling step led to longer shelf-lives of
19 the frozen product, according to sensory analysis. Such result was in agreement with
20 biochemical analyses concerning lipid oxidation, in which higher TBA-i and FR values
21 were obtained for frozen sardines that had been preliminary chilled with flake ice.

22 Since the effect of previous chilling conditions (storage time, fish/ice ratio, room
23 storage temperature) on the quality of frozen fish has been demonstrated (Deng, 1978;
24 Sankar & Viswanathan Nair, 1988; Undeland & Lingnert, 1999; Aubourg et al., 2002),
25 the application of the slurry ice technology as a preliminary processing step prior to

1 freezing and frozen storage is considered a promising strategy to achieve frozen fish
2 products of higher quality.

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4

1

FIGURE LEGENDS

2

3 **Figure 1:** Comparative evolution of the NaCl content (g NaCl / 100g muscle) in frozen

4 sardine muscle subjected to preliminary slurry ice chilling during 2 (■), 5 (■) and 9 (□) days.

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TABLE 1

Scale employed for evaluating quality of frozen sardine

ATTRIBUTE	QUALITY CATEGORIES			
	E (highest quality)	A (good quality)	B (fair quality)	C (rejectable quality)
External Odour	Sharply seaweedy and shellfish	Weakly seaweedy and shellfish	Slightly sour and incipiently rancid	Sharply sour and rancid
Skin and External Colour	Very intense pigmentation; absence of yellowish spots	Insignificant pigmentation losses; absence of yellowish spots	Pigmentation discoloured and without shine; incipient yellowish spots	Important pigmentation losses; presence of yellowish spots
Flesh Appearance	Strongly hydrated and pink; myotomes totally adhered	Still hydrated and pink; myotomes adhered	Slightly dry and pale; myotomes adhered in groups	Yellowish and dry; myotomes totally separated

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8
9

TABLE 2

Comparative evolution of free fatty acid (FFA; g oleic acid / 100g lipids) formation* in frozen sardine muscle subjected to preliminary chilled storage under slurry ice and flake ice**

Chilling Storage Time (days)	Preliminary icing treatment	Frozen Storage Time (months)		
		1	2	4
2	Slurry Ice	0.15 a (0.08)	0.90 b (0.38)	^y 1.99 c (0.55)
	Flake Ice	0.32 a (0.19)	0.74 b (0.10)	^z 1.29 c (0.04)
5	Slurry Ice	^z 0.19 a (0.03)	0.78 b (0.09)	^y 1.60 c (0.52)
	Flake Ice	^y 0.30 a (0.06)	0.86 b (0.25)	^z 1.33 b (0.35)
9	Slurry Ice	0.39 a (0.07)	1.06 b (0.19)	1.34 b (0.29)
	Flake Ice	0.32 a (0.10)	0.93 b (0.25)	1.68 c (0.33)

* Mean values of three independent determinations (n=3) are presented. Standard deviations are indicated in brackets. For each chilling storage time and each frozen storage time, values preceded by different superscripts (y, z) denote significant differences (p<0.05) between both icing conditions. For each chilling storage time and each chilling condition, values followed by different letters denote significant differences (p<0.05) as a result of the frozen storage time.

** Starting raw fish FFA value: 0.09±0.07.

TABLE 3

**Comparative evolution of the peroxide value (PV; meq active oxygen / kg lipids)*
in frozen sardine muscle subjected to preliminary chilled storage under slurry ice
and flake ice****

Chilling Storage Time (days)	Preliminary icing treatment	Frozen Storage Time (months)		
		1	2	4
2	Slurry Ice	^z 1.50 a (0.24)	2.30 a (0.95)	7.57 b (1.12)
	Flake Ice	^y 2.78 a (0.28)	1.90 a (1.18)	6.35 b (2.00)
5	Slurry Ice	^z 2.57 a (1.05)	3.83 a (2.01)	^y 12.20 b (2.95)
	Flake Ice	^y 5.24 a (0.98)	5.61 a (2.39)	^z 5.22 a (2.49)
9	Slurry Ice	8.97 a (2.27)	8.89 a (3.11)	14.60 b (1.43)
	Flake Ice	8.87 a (0.74)	9.66 a (2.11)	18.29 b (3.31)

* Mean values of three independent determinations (n=3) are presented. Standard deviations are indicated in brackets. For each chilling storage time and each frozen storage time, values preceded by different superscripts (y, z) denote significant differences (p<0.05) between both icing conditions. For each chilling storage time and each chilling condition, values followed by different letters denote significant differences (p<0.05) as a result of the frozen storage time.

** Starting raw fish PV: 0.99±0.64.

TABLE 4

Comparative evolution of the thiobarbituric acid index (TBA-i; mg malondialdehyde / kg muscle)* in frozen sardine muscle subjected to preliminary chilled storage under slurry ice and flake ice**

Chilling Storage Time (days)	Preliminary icing treatment	Frozen Storage Time (months)		
		1	2	4
2	Slurry Ice	0.26 a (0.06)	0.56 b (0.20)	1.04 c (0.22)
	Flake Ice	0.38 a (0.11)	0.43 a (0.11)	0.97 b (0.19)
5	Slurry Ice	0.47 a (0.09)	^z 0.58 a (0.17)	^z 1.09 b (0.33)
	Flake Ice	0.53 a (0.17)	^y 1.16 b (0.26)	^y 2.31 c (0.29)
9	Slurry Ice	0.53 a (0.15)	1.00 ab (0.50)	^z 1.36 b (0.46)
	Flake Ice	0.72 a (0.22)	1.18 b (0.04)	^y 3.31 c (0.51)

* Mean values of three independent determinations (n=3) are presented. Standard deviations are indicated in brackets. For each chilling storage time and each frozen storage time, values preceded by different superscripts (y, z) denote significant differences (p<0.05) between both icing conditions. For each chilling storage time and each chilling condition, values followed by different letters denote significant differences (p<0.05) as a result of the frozen storage time.

** Starting raw fish TBA-i: 0.16±0.03.

TABLE 5

Comparative evolution of the fluorescence ratio (FR) value* in frozen sardine muscle subjected to preliminary chilled storage under slurry ice and flake ice**

Chilling Storage Time (days)	Preliminary icing treatment	Frozen Storage Time (months)		
		1	2	4
2	Slurry Ice	0.26 a (0.07)	0.17 a (0.07)	0.32 a (0.08)
	Flake Ice	0.30 b (0.02)	0.18 a (0.03)	0.31 b (0.14)
5	Slurry Ice	0.30 b (0.07)	0.14 a (0.07)	0.27 b (0.05)
	Flake Ice	0.38 b (0.05)	0.20 a (0.06)	0.23 a (0.06)
9	Slurry Ice	0.26 a (0.04)	0.21 a (0.07)	^z 0.31 a (0.09)
	Flake Ice	0.29 a (0.04)	0.25 a (0.03)	^y 0.49 b (0.07)

* Mean values of three independent determinations (n=3) are presented. Standard deviations are indicated in brackets. For each chilling storage time and each frozen storage time, values preceded by different superscripts (y, z) denote significant differences (p<0.05) between both icing conditions. For each chilling storage time and each chilling condition, values followed by different letters denote significant differences (p<0.05) as a result of the frozen storage time.

** Starting raw fish FR value: 0.12±0.05.

TABLE 6

**Sensory assessment* of frozen sardine subjected to preliminary chilled storage
under slurry ice and flake ice**

Chilling Time (days)	Icing Treatment	Frozen Storage Time (months)	Attribute		
			External Odour	Skin and External Colour	Flesh Appearance
2	Slurry Ice	1	A	A	A
		2	A	A	B
		4	B	B	C
	Flake Ice	1	B	A	B
		2	B	B	C
		4	B	B	C
5	Slurry Ice	1	B	A	B
		2	B	A	B
		4	C	B	C
	Flake Ice	1	B	B	B
		2	B	C	C
		4	C	C	C
9	Slurry Ice	1	B	B	B
		2	B	B	B
		4	C	C	C
	Flake Ice	1	C	C	C
		2	C	C	C
		4	C	C	C

* Freshness categories as expressed in the Materials and Methods section. Starting raw fish was category E in all attributes.