Title:
Effect of storage in slurry ice on the quality and shelf life of farmed turbot
(Psetta maxima)

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Short title: Shelf life of turbot stored in slurry ice
ABSTRACT

The application of slurry ice, a binary mixture of small spherical ice crystals surrounded by seawater at subzero temperature, is a potentially new preservation method for farmed turbot (*Psetta maxima*), a flat fish species of increasing commercial interest. Comparative biochemical, microbiological and sensory analyses were carried on turbot specimens stored in either slurry ice or flake ice for up to 40 days. The results obtained in the sensory analysis correlated well with the observed chemical and microbial changes. Storage of turbot in slurry ice resulted in a slowing-down of the nucleotide degradation pathway and lipid oxidation mechanisms. A good stabilization of the high molecular wt protein fraction of turbot muscle was also achieved as a consequence of storage in slurry ice. A slower production of both trimethylamine and total volatile bases was also observed. Likewise, low levels of total aerobes, anaerobes, coliforms, and proteolytic bacteria were attained. According to the biochemical, microbial and sensory analyses, and the statistically-significant (p<0.05) differences observed with respect to data obtained in parallel with conventional icing, the application of slurry ice to farmed turbot is advisable to achieve better quality maintenance during storage and distribution.

**Key Words:** Chilling; Slurry ice; Turbot; Aquaculture; Shelf life; Quality
INTRODUCTION

Marine species deteriorate rapidly after death due to the effect of a wide variety of biochemical and microbial degradation mechanisms. However, the loss of quality depends directly on the nature of fish species and on the handling and storage conditions (Whittle and others 1990; Olafsdóttir and others 1997). Once the fish are caught, on-board storage conditions exert a strong effect on the quality of manufactured fish products and, accordingly, on their commercial value (Piggot and Tucker 1987; Ashie and others 1996). With a view to preserving the greatest proportion of a fish catch in an acceptable manner, several on-board handling systems, including storage in flake ice (Nunes and others 1992), refrigerated seawater (Kraus 1992), or the addition of chemicals (Ponce de Leon and others 1993; Hwang and Regenstein 1995), have been proposed.

Slurry ice, also known as fluid ice, slush ice, liquid ice or flow ice, offers a promising technique for preservation and consists of an ice-water suspension at a subzero temperature. Two main features of slurry ice are its faster chilling rate, deriving from its higher heat-exchange capacity, and the reduced physical damage caused to seafood products by its microscopic spherical particles, as compared with flake ice. The overall covering of the fish surface by the slurry ice mixture also protects the fish from the action of oxygen. The versatility of the slurry ice technique has also been highlighted. Besides being a pumpable mixture, slurry ice may be combined with other additives, such as ozone or melanosis inhibitors (Huidobro and others 2002). However, despite its theoretical advantages few empirical data concerning the practical advantages derived from the use of slurry ice for the storage of marine species are available.

Fish technologists and the fish trade in general are paying increasing attention to aquaculture products as a source of fish and other seafood products (FAO 2000; Josupeit and others 2001). Such is the case of turbot (Psetta maxima, also known as Scophtalmus maximus), a highly valued flat fish species appreciated for its firm, white and flavorful flesh. Recently, increasing production of this species as an aquaculture product has raised its availability. However,
previous research on farmed turbot has mainly focused on farming conditions (Tocher and others 1992; Serot and others 2001), on the sensory differences between wild and farmed turbot (Prost and others 1998), and on the quality changes deriving from high pressure (Chevalier and others 2001), or thermal processing (Madeira and Penfield 1985). More recently, studies on the sensory, microbial and biochemical changes occurring in farmed turbot during refrigerated storage have been initiated (Rodríguez and others 2003; Aubourg and others 2004).

With the aim of having a better understanding about the potential applications of slurry ice technology, in the present work the effect of this advanced storage system on quality losses and the shelf life of farmed turbot was evaluated. The results were compared with a parallel study addressing the use of flake ice. To this end, the evolution of the sensory features, microbial activity, and the modifications affecting the most relevant biochemical components, basically lipids, proteins and nucleotides, was evaluated in farmed turbot stored in either slurry ice or flake ice for 40 days.
MATERIALS AND METHODS

Refrigeration systems

Slurry ice was prepared using a FLO-ICE prototype (Kinarca S.A.U., Vigo, Spain). The composition of the flow 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 and the temperature of the turbot specimens was in the range of -1°C/-1.5°C. Flake ice was prepared using freshwater with an Icematic F100 Compact device (Castelmac SPA, Castelfranco, Italy). The temperature of turbot specimens stored in flake ice was in the range of 0°C/1°C. The fish specimens were surrounded by either slurry ice or flake ice at a fish:ice ratio of 1:1, and stored for up to 40 days in a refrigerated room at 2°C. When required, the ice mixtures were renewed.

Fish material, processing and sampling

Two-year old farmed turbot (Psetta maxima) specimens were obtained from the Stolt Sea Farm, S.A. (Carnota, Galicia, Spain). Fish specimens were sacrificed in a seawater-ice mixture and then kept in ice for 10 h as they arrived at our laboratory. The fish specimens were not headed nor gutted. The length of the fish was 40-45 cm, the width was 29-35 cm, and the weight was 1,400 to 1,700 g. Three different batches were used and studied separately along the whole experimental period for each type of ice. Samples were taken from each batch on days 0, 2, 5, 9, 14, 19, 22, 26, 29, 33, 36 and 40. Once whole fish had been subjected to sensory analysis, the white muscle was separated and homogenized for microbiological and biochemical analyses. All sensory, biochemical and microbiological analyses were performed in triplicate.

Sensory analyses

Sensory analysis was conducted by a sensory panel consisting of five experienced judges, according to traditional guidelines (Table 1) concerning fresh and chilled fish (DOCE 1989). 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 odor, gills, consistency and flesh odor. The scores of the different panelists were averaged.

Microbiological analyses

Samples of 25 g of fish muscle were dissected aseptically from chilled turbot specimens, mixed with 225 ml of peptone water, and homogenized in a stomacher (Seward Medical, London, UK). Serial dilutions from the microbial extracts were prepared in peptone water as previously described (Ben-Gigirey and others 1998, 1999). Total aerobic counts and anaerobes were investigated in Plate Count Agar (PCA, Oxoid Ltd., London, UK) by standard laboratory methods, as previously described (Ben-Gigirey and others 1998, 1999). Lactose-fermenting Enterobacteriaceae (coliforms) were investigated carried out in Violet Red Bile Agar (VRBA medium, Merck, Darmstadt, Germany) following the manufacturer’s instructions. The proteolytic phenotype was investigated in casein-agar medium (Phaff and others 1994), as previously described (Ben-Gigirey and others 2000).

Total volatile basic nitrogen assay by steam distillation

Total volatile base-nitrogen (TVB-N) values were measured by the Antonacopoulos method (Antonacopoulos 1960), with the modifications described elsewhere (Aubourg and others 1997). Briefly, fish muscle (10 g) was extracted with 6% (w/v) perchloric acid and brought up to 50 ml, the TVB-N content being determined, after steam-distillation of the acid extracts rendered alkaline to pH 13 with 2% (w/v) NaOH, by titration of the distillate with 10 mM hydrochloric acid. The results were expressed as mg TVB-N/100 g muscle.

Trimethylamine assay

Trimethylamine-nitrogen (TMA-N) values were obtained by the picrate method, as previously described (Tozawa and others 1971). This involves the preparation of a 5% (w/v) trichloroacetic acid extract of fish muscle (40%). Results were expressed as mg TMA-N/100 g muscle.
**Nucleotide analysis and pH determination**

Nucleotide extracts were prepared according to the method of Ryder (1985) and were stored at -30°C until analysis. Nucleotide analysis was performed by HPLC using a Beckman device provided with the programmable solvent module 126 (Beckman), and the scanning detector module 167 (Beckman) connected to System Gold software, version 8.1 (Beckman). Separations were accomplished on a reverse-phase Spherisorb ODS-2 C$_{18}$ 250 x 4.60 mm column (Waters, Milford, MA), with an internal particle diameter of 5 μm. The composition of the mobile phase was as follows: solvent A was composed of 0.04 M KH$_2$PO$_4$ + 0.006 M K$_2$HPO$_4$, pH 7; solvent B was acetonitrile. The solvents were filtered through a 0.45 μm aqueous filter before use. Separations were carried out using a continuous gradient elution. The eluent was monitored at 254 nm and the running time was 10 min. Standard curves for adenosine 5’-triphosphate (ATP) and each compound involved in its degradation pathway [adenosine 5’-diphosphate (ADP), adenosine 5’-monophosphate (AMP), inosine 5’-monophosphate (IMP), inosine (Ino) and hypoxanthine, (Hx)] were constructed in the 0 to 1 mM range. All nucleotide standards were obtained from the Sigma Chemical Co. (St. Louis, MO). The widely used K value was calculated according to the following concentrations ratio:

$$K\text{ value} = 100 \times \frac{(Hx + Ino)}{(ATP + ADP + AMP + IMP + Ino + Hx)}.$$  

The pH was determined using a 6-mm diameter insertion electrode (Crison, Barcelona, Spain).

**Lipid damage analyses**

The lipid fraction was extracted using the Bligh and Dyer method (Bligh and Dyer 1959). The free fatty acid (FFA) content was determined by the Lowry and Tinsley method, based on complex formation with cupric acetate-pyridine (Lowry and Tinsley 1976). The results were expressed as g FFA/100 g lipids. The formation of interaction compounds was investigated by means of fluorescent properties. To do so, fluorescence formation (Perkin-Elmer LS 3B) at 393/463 nm and 327/415 nm was studied as previously described (Aubourg and others 1997;
Aubourg and others 1998). Relative fluorescence (RF) was calculated as follows: $RF = \frac{F}{F_{st}}$, where $F$ is the fluorescence measured at each excitation/emission maximum, and $F_{st}$ is the fluorescence intensity of a quinine sulfate solution (1 mg/l in 0.05 M H$_2$SO$_4$) at the corresponding wavelength. The fluorescence ratio (FR) was calculated as the ratio between both RF values: $FR = \frac{RF_{393/463nm}}{RF_{327/415nm}}$. The FR value was analyzed in the aqueous phase resulting from the lipid extraction.

**Protein analyses**

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). A quantity of 500 mg of muscle was homogenized for 60 s in 4 ml of the buffer solution, as previously described (Piñeiro and others 1999). 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 Laboratories Inc. Hercules, CA). A standard curve constructed for bovine serum albumin was used as reference.

**Statistical analyses**

Data from the different chemical measurements were subjected to one-way analysis of variance; comparison of means was performed using a least-squares difference (LSD) method (Statsoft 1994). The SPSS software (SPSS Inc., Chicago, IL) was also used to explore the statistical significance of the differences between batches, 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, the turbot specimens stored in slurry ice maintained good quality, being classified in the E or A categories up to day 22 (Table 2). After this time, quality decreased, and by day 33 the turbot stored in slurry ice was no longer acceptable. The main aspects related to quality loss were the gill odor and color. In contrast with these results, the good sensory quality, E and A categories, of farmed turbot specimens stored in flake ice were maintained only up to day 14, such turbot specimens being rejected on day 22. In the case of turbot stored in flake ice, the presence of abundant skin mucus and the external odor were the limiting factors of acceptability. However, and although farmed turbot stored in flake ice has a longer shelf life than other common medium-sized fish species, such as albacore (Pérez-Villareal and Pozo 1990) or hake (Ruiz-Capillas and Moral 2001), the storage of turbot in slurry ice allowed a significant extension of its shelf life, from 14 days to 22 days.

Microbiological analyses

The total aerobic counts only varied significantly (p<0.05) after 19 days of storage in the slurry ice batch, this indicating a slower growth of this microbial group with respect to the flake ice batch, where aerobic counts above $10^6$ CFU/g were determined after 14 days of storage (Figure 1a). A similar type of behavior was observed for proteolytic bacteria (Figure 1b). Thus, the counts of proteolytic bacteria in turbot muscle stored in slurry ice did not reach levels of $10^5$ CFU/g until very advanced storage periods in slurry ice (40 days), these being significantly (p<0.05) lower than those observed in the flake ice batch after only 14 days of storage ($7.5 \times 10^6$ CFU/g). The counts of anaerobes in turbot muscle stored in slurry ice did not vary significantly (p<0.05) along storage, being in all cases below $10^5$ CFU/g. These results clearly indicate the very slow growth of this microbial group in turbot muscle stored in slurry ice. In contrast to the results obtained for slurry ice, the counts of anaerobes in turbot muscle stored in flake ice varied significantly (p<0.05) after 26 days of storage, reaching counts above $3.8 \times 10^4$ CFU/g at that
time. With respect to the development of coliforms in turbot muscle stored in slurry ice, these
did not vary significantly along the 40 days of storage. Thus, it should be remarked that the
average counts of coliforms were below 10 CFU/g, and only reached a level of 12 CFU/g after
36 days of storage in slurry ice. In contrast, turbot muscle stored in flake ice exhibited a
significant (p<0.05) increase in the coliforms counts after 22 days of storage, reaching numbers
close to 10^4 CFU/g after 29 days of storage.

According to the Tukey and Scheffé tests, the results obtained indicated statistically-
significant differences (p<0.05) between batches for all four microbial groups. These findings
clearly indicate a significantly slower growth of the four microbial groups investigated in turbot
muscle subjected to storage in slurry ice as compared with traditional flake ice.

**Biochemical analyses**

No statistically-significant differences were observed in the 0-9 day period for the pH value
of turbot muscle stored in slurry ice (Figure 2). A slight increase in pH was observed in this
batch on day 14. However, the pH value decreased on day 29 but increased after that day up to
the end of the experiment (Figure 2). By contrast, turbot stored in flake ice exhibited pH values
higher than 6.50 and 6.70 after 14 days and 19 days of storage, respectively, these figures being
considerably above the pH values described in this work for turbot stored in slurry ice (Figure
2). As expected from the results obtained, statistical analysis confirmed that the lowest pH
values determined for turbot stored in slurry ice were significantly (p<0.05) below those
exhibited by turbot muscle stored in flake ice. These results indicate a better control of both
endogenous and microbial alkalinising mechanisms in turbot muscle as a consequence of
storage in slurry ice, as compared to storage in flake ice. Unlike the results obtained in this
study, previous reports have also described steady increases in the pH value for other fish
species stored in flake ice (Nunes and others 1992; Ruiz-Capillas and Moral 2001).

Nucleotide degradation along storage was studied in turbot muscle stored in either slurry ice
or flake ice on the basis of the K value (Figure 3). The K value increased in the slurry ice batch
to values close to 40 by day 14, this being followed by a slower gradual increase to a K value
above 50 by day 40. Thus, the K values of the turbot specimens stored in slurry ice proved to be considerably lower than those found in turbot stored in flake ice: in the latter case, the K values determined reached levels higher than 50 and 70 after 9 days and 14 days of storage, respectively (Figure 3). Statistical analysis confirmed that the rate of nucleotide degradation, as determined by the K value, was significantly (p<0.05) lower for turbot stored in slurry ice than for turbot stored in flake ice, this indicating a significant slowing down of the autolytic degradation events of turbot muscle in the former batch.

The TVB-N content of turbot stored in slurry ice showed very slight differences along the storage time (Figure 4). Thus, the TVB-N content of turbot stored in slurry ice showed a decreasing trend up to day 22, this being followed by an increasing trend up to the end of the storage period. In all cases, the TVB-N content of turbot stored in slurry ice was very low. By contrast, turbot stored in flake ice exhibited TVB-N values above 30 mg/100 g after 33 days of storage (Figure 4). Statistical analysis revealed that the storage of turbot in slurry ice involved a significantly (p<0.05) slower formation of TVB-N in comparison with storage in flake ice. Unlike the results obtained in our study for turbot stored in slurry ice, other authors have also reported sharp increases in the TVB-N content of medium-sized fish species, haddock and hake, after 9-12 days of storage in flake ice, this coinciding with the end of the microbial lag phase (Fernández-Salgueiro and others 1987; Ruíz-Capillas and Moral 2001; Baixas-Nogueras and others 2002).

The TMA-N index of turbot muscle increased very slowly in the period between 0-26 days of storage in slurry ice (Figure 5). After this time, a sharp increase was observed between days 26 and 29, this being followed by a slower increase up to the end of storage. Average TMA-N values below 0.4 mg/100 g and 0.5 mg/100 g were obtained for turbot stored in slurry ice on days 22 and 26 of storage, respectively. In contrast, average levels above 1.1 mg/100 g and 1.8 mg/100 g were found in turbot muscle stored for 22 days and 26 days in flake ice, respectively (Figure 5). As expected from the results obtained in the present study, the formation of TMA-N in turbot stored in slurry ice proved to be significantly (p<0.05) lower than in turbot stored in flake ice. Unlike the results obtained in our study for turbot in slurry ice, other reports
concerning small and medium-sized fish species have described sharp increases in TMA-N contents after 9-12 days of storage in flake ice (Pérez-Villarreal and Pozo 1990; Fernández-Salguero and others 1987; Ruíz-Capillas and Moral 2001; Baixas-Nogueras and others 2002).

Lipid hydrolysis occurred along the storage of turbot in slurry ice. In this sense, slight differences were observed as time progressed, as determined by the FFA content (Figure 6). In comparison with the initial value of 0.60 g/100 g lipids at day 0, a notable increase to a FFA content of 2.5 g/100 g lipids was observed on day 29, although the final FFA concentration was below 1.6 g/100 g lipids (Figure 6). The rate of lipid hydrolysis in the slurry ice batch proved to be quite similar to that observed in turbot stored in flake ice. However, in the later batch final concentrations close to 2.5 g/100 g lipids were determined after 40 days of storage (Figure 6). As expected from the results obtained, statistical analysis confirmed that slurry ice did not allow any significant reduction in the rate of lipid hydrolysis in turbot muscle (p<0.05) as compared with flake ice. However, it should be noted that turbot, either stored in flake ice or in slurry ice, exhibits a very low rate of lipid hydrolysis in comparison with common fish species such as sardine (Aubourg and others 1997), horse mackerel (Aubourg 2001), and blue whiting (Aubourg and others 1998).

Lipid oxidation was investigated through the formation of fluorescent compounds. The FR showed a gradual increase along the storage time of turbot in slurry ice (Figure 7). As compared with the initial value, a significant (p<0.05) increase was observed in this parameter on day 5, followed by an increasing trend up to the end of the storage period. In our hands, FR values were never higher than 2, even after 40 days of storage in slurry ice, while FR values higher than 6 were determined at day 19 in turbot stored in flake ice, these results indicating a remarkable inhibition of lipid oxidation mechanisms in the slurry ice batch. Statistical analysis at the p<0.05 level confirmed the inhibitory effect of slurry ice on the formation of fluorescent compounds in turbot muscle, as compared with flake ice.

With respect to the results of protein extractability from turbot muscle in the low ionic-strength buffer used, a significantly high extractability was observed at all sampling times in the slurry ice batch, even after advanced periods of storage (Figure 8). These results are clearly
higher than the extractability values obtained for turbot muscle when storage was carried out in flake ice (Figure 8). Moreover, a better stabilization of the protein fraction above 94 kDa was achieved in turbot muscle subjected to storage in slurry ice as compared with flake ice (data not shown). Previous reports by other authors have proposed that the stabilization of myofibrilar proteins is directly related to better fish quality (Pérez-Villareal and Pozo 1990; Martínez and others 2001). These observations agree with the results obtained in this work. Thus, in comparison with turbot stored in flake ice, the storage of turbot specimens in slurry ice implies a better maintenance of protein extractability and stability even during advanced periods of storage, this coinciding with a better maintenance of texture as determined by sensory analysis.
CONCLUSIONS

In sum, the storage of farmed turbot in slurry ice allowed a remarkably good maintenance of sensory, microbiological and biochemical quality, involving an extension of its shelf life as compared with storage in flake ice. The lower counts of total aerobes, anaerobes, coliforms and proteolytic bacteria, and a good maintenance of pH were achieved. Biochemical analyses pointed to very low TMA-N and TVB-N contents and significantly low rates for both the nucleotide degradation pathway, as determined by the K value, and lipid oxidation mechanisms, as determined by the measurement of fluorescent compounds. Furthermore, a greater stability of high molecular wt proteins was observed along storage. On the basis of the results obtained, the use of slurry ice for the storage of farmed turbot is encouraged owing to the better maintenance of quality and the extended shelf life deriving from its use in comparison with flake ice.
This work was supported by a project granted by the Secretaría Xeral de I+D from the Xunta de Galicia (Project PGIDT01MAR40202PR). The authors wish to thank KINARCA S.A.U. for providing the slurry ice equipment. The authors also thank Alicia Ojea, Marcos Trigo, José M. Antonio and Lorena Barros for technical assistance and the Stolt Sea Farm S.A. (Carnota, Galicia, Spain) for providing the turbot specimens.
REFERENCES


Table 1 – Scale employed for evaluating freshness of chilled turbot.

<table>
<thead>
<tr>
<th>Attribute</th>
<th>Highest quality (E)</th>
<th>Good quality (A)</th>
<th>Fair quality (B)</th>
<th>Unacceptable (C)</th>
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<tbody>
<tr>
<td>Skin aspect</td>
<td>Transparent mucus; very intense pigmentation</td>
<td>Milky mucus; insignificant pigmentation losses</td>
<td>Slightly grayish mucus; pigmentation without shine</td>
<td>Widely opaque mucus; important pigmentation losses</td>
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<td>External odor</td>
<td>Sharply seaweedy and shellfish</td>
<td>Weakly seaweedy and shellfish</td>
<td>Slightly sour and putrid</td>
<td>Sharply sour and putrid</td>
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<tr>
<td>Gills</td>
<td>Brightly red; without odor; lamina perfectly separated</td>
<td>Rose colored; without odor; lamina adhered in groups</td>
<td>Slightly pale; fishy odor; lamina adhered in groups</td>
<td>Grey-yellowish color; intense ammonia odor; lamina totally adhered</td>
</tr>
<tr>
<td>Consistency</td>
<td>Presence or partial disappearance of rigor mortis symptoms</td>
<td>Firm and elastic; pressure signs disappear completely</td>
<td>Elasticity notably reduced; presence of mechanical signs</td>
<td>Important shape changes due to mechanical factors</td>
</tr>
<tr>
<td>Flesh odor</td>
<td>Sharply seaweedy and shellfish</td>
<td>Weakly seaweedy and shellfish</td>
<td>Slightly sour and putrid</td>
<td>Sharply sour and putrid</td>
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Table 2 – Sensory evaluation of farmed turbot during its chilled storage.

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Note: E = Excellent, A = Acceptable, B = Below acceptable, C = Critical
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LEGENDS TO FIGURES

Figure 1. Comparative evolution of the aerobes (A) and proteolytic bacteria (B) in turbot muscle during storage in slurry ice (black box) or flake ice (white box). Results are expressed as log CFU/g.

Figure 2. Comparative evolution of the pH value in turbot muscle during storage in slurry ice (black box) or flake ice (white box).

Figure 3. Comparative evolution of the nucleotide degradation rate, as determined by the K value, in turbot muscle during storage in slurry ice (black box) or flake ice (white box). Results are expressed as the ratio of nucleotide concentrations described in the text.

Figure 4. Comparative formation of total volatile base-nitrogen (TVB-N) in turbot muscle during storage in slurry ice (black box) or flake ice (white box). Concentrations are expressed as mg TVB-N/100 g muscle.

Figure 5. Comparative formation of trimethylamine-nitrogen (TMA-N) in turbot muscle during storage in slurry ice (black box) or flake ice (white box). Concentrations are expressed as mg TMA-N/100 g muscle.

Figure 6. Comparative release of free fatty acids (FFA) in turbot muscle during storage in slurry ice (black box) or flake ice (white box). Concentrations are expressed as g FFA/100 g lipids.

Figure 7. Comparative lipid oxidation, as determined by the formation of interaction compounds, in turbot muscle during storage in slurry ice (black ice) or flake ice (white box). The fluorescence ratio (FR) is calculated as described in the text.
Figure 8. Comparative evolution of protein extractability in turbot muscle during storage in slurry ice (black ice) or flake ice (white box). The results are expressed are g protein/100 g muscle.
FIGURE 1

A

![Graph showing the change in Aerobes (log CFU/g) over storage time (days)].

B

![Graph showing the change in Proteolytic bacteria (log CFU/g) over storage time (days)].
FIGURE 2
FIGURE 3
FIGURE 4

[Bar chart showing TVB-N (mg/100 g) vs storage time (days). The x-axis represents storage time in days (0, 2, 5, 9, 14, 19, 22, 26, 29, 33, 36, 40) and the y-axis represents TVB-N in mg/100 g (0 to 50). The bars indicate the mean values with error bars representing the standard deviation.]
FIGURE 5

TMA-N (mg/100 g) vs. Storage time (days)
FIGURE 6

![Graph showing FFA (%) over storage time (days)]
FIGURE 7