Quality retention during the chilled distribution of farmed turbot (*Psetta maxima*): Effect of a primary slurry ice treatment

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SUMMARY

The quality and shelf-life of chilled farmed turbot (*Psetta maxima*) was evaluated by sensory, microbiological and biochemical procedures after being subjected to a two-step refrigeration storage in slurry ice and flake ice, respectively. Turbot specimens were stored for 10 or 17 days in slurry ice, and then were transferred to flake ice for 1 to 3 days to simulate the sale conditions in the market. The results were compared with control batches stored only in flake ice and processed in parallel. Storage of turbot in the two-step strategy resulted in a better maintenance of sensory quality, especially with regard to its mucus production and gill odour development, better control of microbial activity, especially of aerobes, and the slowing down of some biochemical degradation mechanisms such as the nucleotide degradation pathway and trimethylamine production. As a consequence, the shelf-life was extended significantly. From these results it can be concluded that the refrigerated transport of farmed turbot in slurry ice enhances its shelf-life, before its transfer into flake ice in the retail market.

Running Title: Distribution of chilled farmed turbot

Key words: Turbot, farming, chilling, slurry ice, quality, distribution
INTRODUCTION

Aquatic food products deteriorate rapidly post-mortem due to the effects of a variety of degradation mechanisms (Cheftel & Cheftel, 1976; Pigott & Tucker, 1987; Sikorski & Bonnie, 1994). Among these, microbial spoilage and the biochemical degradation of non-protein-nitrogen compounds and proteins, with the subsequent formation of a variety of products such as hypoxanthine, trimethylamine, among others, should be highlighted as being accurate indices of quality loss. Products from fish whose temperature varies with the environment, have a high water content, a soft muscular and skin structure, and a low collagen content (Brown, 1986). Therefore these products should be considered as among the most perishable foods, thus requiring a rapid and thorough cooling step after harvesting to preserve their quality in the fresh state. Other steps included before consumption are slaughtering –of both farmed and wild species–, storage, long-distance transport by ship, airplane or lorry, and every step involved in local distribution and domestic storage.

Flake ice has traditionally been the preferred system to cool fresh aquatic food products to final temperatures slightly above 0°C (Heen, 1982; Whittle et al., 1990; Ashie et al., 1996). Recently, newer chilling systems have enabled the storage of aquatic food products at subzero temperatures through the addition of salts and other compounds to ice-water mixtures (Chapman, 1990; Harada, 1991; Yamada et al., 2002). These are called “slurry ice systems”, “water-binary systems” or “two-phase aqueous secondary refrigerants” since they comprise two different phases –liquid (water) and solid (ice). The main features of slurry ice are (i) storage at a temperature below 0°C, (ii) a faster chilling rate, due to the higher heat-exchange capacity, (iii) the reduced physical damage caused by the spherical microscopic particles characteristic of slurry
ice, as compared with flake ice, and (iv) the complete covering of the fish surface by the slurry ice mixture protecting the fish from the action of oxygen, which also helps to prevent deterioration through oxidation and dehydration.

Spain, and particularly its North-western area, is the major European producer and exporter of farmed turbot, a flatfish species of high commercial value (Person-Le Ruyet, 1990; FAO, 2002). However, this fish species has to be stored for moderately long time periods during its transport to other countries. In spite of the increasing tendency to transport this fish species in slurry ice, once the destination point is reached, farmed turbot specimens are sold in the retail market surrounded by flake ice. This final step can last from 1 to 3 days. The effects of storage in slurry ice on the quality of several fish species has been studied by several authors (Price et al., 1991; Chinivasagam et al., 1998; Huidobro et al., 2001; Losada et al., 2004). However the effects of a rapid temperature change suffered by fish when it is changed from storage in slurry ice to flake ice (from -1.5°C to +0.5°C) in the retail markets has not been studied up to now. Therefore, the aim of the present work was to study the effect of a two-step storage strategy –storage in slurry ice followed by storage in flake ice– on the sensory, biochemical and microbial quality of farmed turbot, compared with samples stored all the time using flake ice alone. Comparison to farmed turbot samples stored all the time under flake ice is carried out.
MATERIALS AND METHODS

Refrigeration systems

In this work, a slurry ice prototype (FLO-ICE\textsuperscript{TM}, Kinarca S.A.U., Vigo, Spain) was used. The composition of the liquid-ice binary mixture was 40% ice/60% water, prepared from filtered seawater. The temperature of the liquid-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 an equal weight of slurry or flake ice, and stored in a refrigerated room at 2°C. When required, the flake ice and the slurry ice mixture were renewed.

Fish material and sampling

Two-year old farmed turbot (\textit{Psetta maxima}) specimens were obtained from Stolt Sea Farm, S.A. (Carnota, Spain). Fish specimens were slaughtered in a water-ice mixture and then kept in ice until they arrived at our laboratory (6 hours). The fish specimens were not headed or gutted. The length of the fish was in the 39-46 cm range, while the width was in the 29-35 cm range; the weight range was 1.600-1.900 kg.

Three fresh specimens were studied at day 0 as starting material, while 18 specimens were divided into two groups of nine specimens that were surrounded by slurry ice at a 1:1 ratio. After 10 and 17 days of refrigeration, respectively, three specimens of each group were analysed, while the remaining six specimens from each group, as 2 groups of three fish, were transferred into flake ice for 1 and 3 days, respectively, to simulate conditions in the retail market. A control batch (18 turbot specimens) stored in flake ice for the whole experiment was studied in parallel. All analyses were carried out in triplicate.
Sensory analysis

This was conducted by a taste panel consisting of five experienced judges, based on traditional guidelines for fresh and chilled fish (Table 1; DOCE, 1989). Skin, eyes, gills and muscle were evaluated in four categories: A (extra), B (good), C (acceptable) and D (unacceptable). Once the fish had been subjected to sensory analyses the white muscle was separated and homogenised to obtain extracts for microbiological and biochemical analyses.

Microbiological analysis

Samples of 25 g of fish muscle were dissected aseptically from chilled turbot specimens, mixed with 225 ml of peptone water, and homogenised in a stomacher (Seward Medical, London, UK). Serial dilutions from the microbial extracts were prepared in peptone water as previously described (Ben-Gigirey et al., 1998; Ben-Gigirey et al., 1999). Total aerobic counts and anaerobes were determined on Plate Count Agar (PCA, Oxoid Ltd., London, UK) by standard laboratory methods, as previously described (Ben-Gigirey et al., 1998, 1999). Lactose-fermenting Enterobacteriaceae (coliforms) were enumerated using Violet Red Bile Agar (VRBA medium, Merck, Darmstadt, Germany) following the manufacturer’s instructions. Results are expressed in all cases as log CFU g⁻¹.

Biochemical analyses
Total volatile base-nitrogen (TVB-N) values were measured by the Antonacopoulos (1960) method, with the modifications described elsewhere (Aubourg et al., 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 are expressed as mg TVB-N kg\(^{-1}\) muscle.

Trimethylamine-nitrogen (TMA-N) values were obtained by the picrate method, as previously described (Tozawa et al., 1971). This involves the preparation of a 5% (w/v) trichloroacetic acid (TCA) extract of fish muscle (5 g fish muscle / 50 ml TCA). Results are expressed as mg TMA-N kg\(^{-1}\) muscle.

Nucleotide extracts were prepared according to the method of Ryder (1985) and were stored at -30°C until analysis. Analysis was carried out according to Aubourg et al. (2005). The K value was calculated according to the following molar concentrations ratio, where the different molecules involved in the adenosinetriphosphate degradation are included: 

\[
K \text{ value} = 100 \times \frac{\text{hypoxanthine} + \text{inosine}}{\text{adenosinetriphosphate} + \text{adenosinediphosphate} + \text{adenosinemonophosphate} + \text{inosinemonophosphate} + \text{inosine} + \text{hypoxanthine}}.
\]

The lipid fraction was extracted using the Bligh & Dyer (1959) method. The free fatty acid (FFA) content was determined on the lipid extract by the Lowry & Tinsley (1976) method, based on complex formation with cupric acetate-pyridine. The results are expressed as g FFA kg\(^{-1}\) lipids.

Lipid oxidation was measured according to the interaction compound formation (Pokorný, 1981; Howell, 1995). This was studied by fluorescence assessment (Perkin-Elmer LS 3B) at 393/463 nm and 327/415 nm as previously described (Aubourg, 1999).
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 sulphate solution (1 mg l\(^{-1}\) 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 analysed in the aqueous phase resulting from the lipid extraction.

**Statistical analyses**

Data from the different chemical measurements were subjected to one-way analysis of variance with comparison of means performed using a least-significant difference (LSD) method (Statsoft, 1994). SPSS software (SPSS Inc., Chicago, IL, USA) 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**

The study of the sensory quality (Table 2) indicated that the turbot specimens stored in slurry ice for 10 or 17 days maintained a very high (E) or good (A) sensory quality in all parameters evaluated. In contrast, the turbot specimens stored in flake ice for 17 days obtained a B (acceptable) score in five of the seven external parameters evaluated (Table 2), in agreement with previous research carried out by our group on
the same fish species (Rodríguez et al., 2003). The present results indicated that storage in slurry ice resulted in a better maintenance of the sensory quality of farmed turbot as compared with flake ice, in agreement with previous research concerning other fish species (Huidobro et al., 2002; Rodríguez et al., 2004).

The main goal of the sensory studies was to evaluate the effects of the transition from slurry ice to flake ice as the storage medium for farmed turbot. From Table 2 it can be observed that turbot specimens that had been kept continuously in slurry ice for 10 days, maintained a better quality after 1 and 3 days according to skin mucus and gills colour than their counterparts that had been kept in flake ice. Samples from both treatments were still acceptable after 3 days in flake ice.

In the case of the batch that was stored for 17 days in slurry ice, the sensory quality remained good (A) or acceptable (B) after 1 and 3 days of additional storage in flake ice (Table 2). In contrast, the corresponding specimens stored in flake ice merited a C (unacceptable) score in four of the seven parameters after 1 additional day in flake ice and were considered unacceptable according to all the parameters after 3 days in flake ice. These results clearly indicate a significantly better maintenance of sensory quality in the turbot batches that were subjected to a two-step refrigeration strategy. Thus, keeping turbot specimens in slurry ice during transport is advisable even when such specimens are later transferred to a flake ice system similar to those used in the retail market.

**Microbiological analyses**

The turbot specimens stored for 10 days in flake ice or slurry ice exhibited very low microbial numbers for the three microbial groups investigated (Table 3). However, when the turbot specimens stored in slurry ice were transferred to flake ice, the numbers
of the aerobic mesophiles increased up to levels close to $10^5$ CFU g$^{-1}$ after 3 days of storage in flake ice, although these numbers were significantly lower (p<0.05) than in the control batch stored for 13 days in flake ice. For both previous treatments, it should be noted that the microbial concentrations were quite low in all turbot specimens, according to previous research on flake ice stored turbot (Rodríguez et al., 2003).

In contrast, when the experiment was extended to 17 days of storage, more larger differences were observed between treatments (Table 3). Thus, the concentration of aerobes reached levels greater than $10^7$ CFU g$^{-1}$ after 20 days of storage in flake ice, while numbers below $10^4$ CFU g$^{-1}$ were observed in the batch stored for 17 days in slurry ice and then maintained in flake ice for another 3 days. Although the statistical analysis of the anaerobes and coliforms also showed differences between treatments significant at the p<0.05 level, the slurry batch always exhibiting lower microbial concentrations, the numbers were in all cases below $10^4$ CFU g$^{-1}$.

The results of the microbiological analyses clearly showed that the transition from slurry ice to flake ice was accompanied by a notable increase in the microbial numbers. However, the microbial activity of the turbot specimens kept in flake ice up to 3 days after a first refrigeration step in slurry ice of either 10 or 17 days was significantly lower (p<0.05) than in the case of the corresponding batches stored only in flake ice. Accordingly, the two-step refrigeration strategy evaluated in this work yielded a fish with lower bacterial numbers and a higher microbial quality than the traditional strategy of storage in flake ice for the whole time.

**Biochemical analyses**

With reference to biochemical analyses (Table 4), TVB-N and FFA values did not increase as a result of chilled storage time, or produced significant differences
(p>0.05) as a result of the kind of ice previously employed. Neither the total volatile amine formation or lipid hydrolysis development did not indicate differences between both previous treatments.

Fluorescence formation (FR index) showed a significant increase (p<0.05) as a result of chilled storage time, leading to a greater degradation due to oxidation (Aubourg et al., 1998; Losada et al., 2004); however, no significant differences (p>0.05) were found when comparing the two kinds of ice conditions employed as previous treatments.

However, TMA-N and K value determinations led to statistically significant differences in the same way as the sensory and microbiological evaluations. Thus, the two indices increased with chilled storage time and showed significant differences (p<0.05) between treatments, the slurry ice exhibiting lower values. These conclusions agree with previous research where both damage pathways, trimethylamine formation and nucleotide degradation are known to be very accurate methods assessing fish damage during chilled storage (Olafsdóttir et al., 1997).

According to sensory, microbiological and biochemical quality assessments, it seems evident that transport in slurry ice system improves the shelf-life of farmed turbot even when this kind of fish is subsequently transferred to traditional ice and maintained at a 2°C room temperature in the retail market. Results confirm the practical advantages for fish industry of using slurry ice as a chilled storage method to inhibit quality loss during fish processing and retain sensory and nutritional properties. The fact that slurry ice has also been shown to be an advantageous slaughtering method (Huidobro et al., 2001) provides an additional reason for employing this binary technology for transport from fish farms to retail markets.
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Table 1:
Scale employed for evaluating the sensory quality 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 quality (C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Skin</td>
<td>Sharp seaweed and shellfish odour; transparent mucus</td>
<td>Weak seaweed and shellfish odour; milky mucus</td>
<td>Incipiently sour and putrid odour; slightly greyish mucus</td>
<td>Sour and putrid odour; widely opaque mucus</td>
</tr>
<tr>
<td>Eyes</td>
<td>Convex; transparent cornea; bright black pupil</td>
<td>Convex and slightly sunken; slightly opalescent cornea; black cloudy pupil</td>
<td>Flat; opalescent cornea; opaque pupil</td>
<td>Concave and milky cornea; by internal organs blurred</td>
</tr>
<tr>
<td>Gills</td>
<td>Without odour; brightly red</td>
<td>Without odour; rose coloured</td>
<td>Incipient fishy odour; slightly pale</td>
<td>Intense ammonia odour; grey-yellowish colour</td>
</tr>
<tr>
<td>Muscle</td>
<td>Sharp seaweed and shellfish odour; bright-red blood spots</td>
<td>Weak seaweed and shellfish odour; darker red blood spots</td>
<td>Incipiently sour and putrid odour; brown-red blood spots</td>
<td>Sour and putrid odour; brown blood spots</td>
</tr>
</tbody>
</table>
**TABLE 2**

Sensory assessment* of chilled turbot stored in each of the refrigeration strategies evaluated**

<table>
<thead>
<tr>
<th>Chilled storage time (days)</th>
<th>Skin</th>
<th>Eyes</th>
<th>Gills</th>
<th>Muscle</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Odour</td>
<td>Mucus</td>
<td>Odour</td>
<td>Colour</td>
</tr>
<tr>
<td></td>
<td>F1</td>
<td>SI</td>
<td>F1</td>
<td>SI</td>
</tr>
<tr>
<td>10</td>
<td>A</td>
<td>A</td>
<td>E</td>
<td>A</td>
</tr>
<tr>
<td>(+ 1 day in FI)</td>
<td>A</td>
<td>A</td>
<td>B</td>
<td>A</td>
</tr>
<tr>
<td>10 (+ 3 days in FI)</td>
<td>B</td>
<td>B</td>
<td>A</td>
<td>B</td>
</tr>
<tr>
<td>17</td>
<td>B</td>
<td>A</td>
<td>B</td>
<td>A</td>
</tr>
<tr>
<td>(+ 1 day in FI)</td>
<td>C</td>
<td>B</td>
<td>A</td>
<td>B</td>
</tr>
<tr>
<td>(+ 3 days in FI)</td>
<td>C</td>
<td>B</td>
<td>C</td>
<td>B</td>
</tr>
</tbody>
</table>

* Freshness categories: E (excellent), A (good), B (fair) and C (unacceptable). All fish were Category E for all attributes initially.

** Ten and seventeen days in flake ice (FI) and slurry ice (SI) were followed by 0, 1 and 3 days in FI.
<table>
<thead>
<tr>
<th>Chilled storage time (days)</th>
<th>Aerobes (log CFU g⁻¹)</th>
<th>Anaerobes (log CFU g⁻¹)</th>
<th>Coliforms (log CFU g⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>FI</td>
<td>SI</td>
<td>FI</td>
</tr>
<tr>
<td>10</td>
<td>1.58 ± 1.43 b</td>
<td>0.00 ± 0.00 a</td>
<td>0.00 ± 0.00</td>
</tr>
<tr>
<td>10 (+ 1 day in FI)</td>
<td>3.50 ± 0.40 b</td>
<td>2.81 ± 0.32 a</td>
<td>0.00 ± 0.00</td>
</tr>
<tr>
<td>10 (+ 3 days in FI)</td>
<td>5.76 ± 0.37 b</td>
<td>4.71 ± 0.20 a</td>
<td>1.58 ± 0.57 b</td>
</tr>
<tr>
<td>17</td>
<td>4.75 ± 0.77 b</td>
<td>3.16 ± 0.28 a</td>
<td>1.32 ± 0.28 b</td>
</tr>
<tr>
<td>17 (+ 1 day in FI)</td>
<td>5.03 ± 0.44 b</td>
<td>3.00 ± 0.00 a</td>
<td>3.21 ± 0.54</td>
</tr>
<tr>
<td>17 (+ 3 days in FI)</td>
<td>7.11 ± 0.43 b</td>
<td>3.55 ± 0.95 a</td>
<td>3.47 ± 0.32 b</td>
</tr>
</tbody>
</table>

* Mean values (n = 3) followed by standard deviations are indicated. For each kind of analysis, values followed by different letters are significantly (p<0.05) different between FI and SI treatments. Initial fish values: 0.80 ± 1.39 (aerobes), 0.43 ± 0.75 (anaerobes) and 0.00 ± 0.00 (coliforms).

** Refrigeration strategies with flake ice (FI) and slurry ice (SI) as explained in Table 2.
**TABLE 4**

Biochemical analyses* of chilled turbot stored in each of the refrigeration strategies evaluated**

<table>
<thead>
<tr>
<th>Chilled storage time (days)</th>
<th>TVB-N (mg TVB-N kg⁻¹)</th>
<th>TMA-N (mg TMA-N kg⁻¹)</th>
<th>K value</th>
<th>FFA (g FFA kg⁻¹ lipids)</th>
<th>FR</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>FI</td>
<td>SI</td>
<td>FI</td>
<td>SI</td>
<td>FI</td>
</tr>
<tr>
<td>10</td>
<td>213.5 ± 33.4</td>
<td>234.5 ± 47.3</td>
<td>0.60 ± 0.05</td>
<td>0.61 ± 0.09</td>
<td>40.7 ± 4.5 b</td>
</tr>
<tr>
<td>(± 1 day in FI)</td>
<td>221.1 ± 20.3</td>
<td>206.5 ± 33.4</td>
<td>0.68 ± 0.07</td>
<td>0.53 ± 0.08</td>
<td>47.0 ± 5.4 b</td>
</tr>
<tr>
<td>10 (± 3 days in FI)</td>
<td>210.0 ± 3.5</td>
<td>225.8 ± 8.8</td>
<td>0.79 ± 0.06</td>
<td>0.66 ± 0.12</td>
<td>57.0 ± 6.9 b</td>
</tr>
<tr>
<td>17</td>
<td>196.0 ± 12.6</td>
<td>211.8 ± 15.0</td>
<td>3.47 ± 1.51 b</td>
<td>0.81 ± 0.07 a</td>
<td>60.7 ± 2.0 b</td>
</tr>
<tr>
<td>(± 1 day in FI)</td>
<td>187.8 ± 9.6</td>
<td>197.2 ± 13.6</td>
<td>2.81 ± 1.13 b</td>
<td>0.77 ± 0.17 a</td>
<td>63.3 ± 1.2 b</td>
</tr>
<tr>
<td>17 (± 3 days in FI)</td>
<td>217.0 ± 6.3</td>
<td>222.8 ± 14.0</td>
<td>4.44 ± 3.63</td>
<td>2.82 ± 2.70</td>
<td>60.8 ± 4.1</td>
</tr>
</tbody>
</table>

* Abbreviations: TVB-N (total volatile base-nitrogen), TMA-N (trimethylamine-nitrogen), FFA (free fatty acids) and FR (fluorescence ratio). Mean values (n = 3) followed by standard deviations are indicated. For each kind of analysis, values followed by different letters are significantly (p<0.05) different between FI and SI treatments. Initial fish values: 236.3 ± 5.5 (TVB-N), 0.50 ± 0.04 (TMA-N), 3.3 ± 0.4 (K value), 7.61 ± 0.53 (FFA) and 0.36 ± 0.21 (FR).

** Refrigeration strategies with flake ice (FI) and slurry ice (SI) as explained in Table 2.