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5 Quality retention during the chilled distribution of farmed  
6 turbot (*Psetta maxima*): Effect of a primary slurry ice  
7 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

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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.

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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

1 ice, as compared with flake ice, and (iv) the complete covering of the fish surface by the  
2 slurry ice mixture protecting the fish from the action of oxygen, which also helps to  
3 prevent deterioration through oxidation and dehydration.

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

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## MATERIALS AND METHODS

### **Refrigeration systems**

In this work, a slurry ice prototype (FLO-ICE<sup>TM</sup>, 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 (*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.

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## 2 **Sensory analysis**

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

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## 10 **Microbiological analysis**

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

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## 24 **Biochemical analyses**

1 Total volatile base-nitrogen (TVB-N) values were measured by the  
2 Antonacopoulos (1960) method, with the modifications described elsewhere (Aubourg  
3 et al., 1997). Briefly, fish muscle (10 g) was extracted with 6% (w/v) perchloric acid  
4 and brought up to 50 ml, the TVB-N content being determined, after steam-distillation  
5 of the acid extracts rendered alkaline to pH 13 with 2% (w/v) NaOH, by titration of the  
6 distillate with 10 mM hydrochloric acid. The results are expressed as mg TVB-N kg<sup>-1</sup>  
7 muscle.

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

12 Nucleotide extracts were prepared according to the method of Ryder (1985) and  
13 were stored at -30°C until analysis. Analysis was carried out according to Aubourg et al.  
14 (2005). The K value was calculated according to the following molar concentrations  
15 ratio, where the different molecules involved in the adenosinetriphosphate degradation  
16 are included:  $K \text{ value} = 100 \times (\text{hypoxanthine} + \text{inosine}) / (\text{adenosinetriphosphate} +$   
17  $\text{adenosinediphosphate} + \text{adenosinemonophosphate} + \text{inosinemonophosphate} + \text{inosine} +$   
18  $\text{hypoxanthine}).$

19 The lipid fraction was extracted using the Bligh & Dyer (1959) method. The free  
20 fatty acid (FFA) content was determined on the lipid extract by the Lowry & Tinsley  
21 (1976) method, based on complex formation with cupric acetate-pyridine. The results  
22 are expressed as g FFA kg<sup>-1</sup> lipids.

23 Lipid oxidation was measured according to the interaction compound formation  
24 (Pokorný, 1981; Howell, 1995). This was studied by fluorescence assessment (Perkin-  
25 Elmer LS 3B) at 393/463 nm and 327/415 nm as previously described (Aubourg, 1999).

1 Relative fluorescence (RF) was calculated as follows:  $RF = F/F_{st}$ , where F is the  
2 fluorescence measured at each excitation/emission maximum, and  $F_{st}$  is the fluorescence  
3 intensity of a quinine sulphate solution ( $1 \text{ mg l}^{-1}$  in  $0.05 \text{ M H}_2\text{SO}_4$ ) at the corresponding  
4 wavelength. The fluorescence ratio (FR) was calculated as the ratio between both RF  
5 values:  $FR = RF_{393/463\text{nm}} / RF_{327/415\text{nm}}$ . The FR value was analysed in the aqueous phase  
6 resulting from the lipid extraction.

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## 8 **Statistical analyses**

9 Data from the different chemical measurements were subjected to one-way  
10 analysis of variance with comparison of means performed using a least-significant  
11 difference (LSD) method (Statsoft, 1994). SPSS software (SPSS Inc., Chicago, IL,  
12 USA) was also used to explore the statistical significance of the differences between  
13 batches, this including multivariate contrasts and multiple comparisons by the Scheffé  
14 and Tukey tests. A confidence interval at the 95% level ( $p < 0.05$ ) was considered in all  
15 cases.

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## **RESULTS AND DISCUSSION**

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### 20 **Sensory analyses**

21 The study of the sensory quality (Table 2) indicated that the turbot specimens  
22 stored in slurry ice for 10 or 17 days maintained a very high (E) or good (A) sensory  
23 quality in all parameters evaluated. In contrast, the turbot specimens stored in flake ice  
24 for 17 days obtained a B (acceptable) score in five of the seven external parameters  
25 evaluated (Table 2), in agreement with previous research carried out by our group on



1 the same fish species (Rodríguez et al., 2003). The present results indicated that storage  
2 in slurry ice resulted in a better maintenance of the sensory quality of farmed turbot as  
3 compared with flake ice, in agreement with previous research concerning other fish  
4 species (Huidobro et al., 2002; Rodríguez et al., 2004).

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

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

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## 22 **Microbiological analyses**

23 The turbot specimens stored for 10 days in flake ice or slurry ice exhibited very  
24 low microbial numbers for the three microbial groups investigated (Table 3). However,  
25 when the turbot specimens stored in slurry ice were transferred to flake ice, the numbers

1 of the aerobic mesophiles increased up to levels close to  $10^5$  CFU g<sup>-1</sup> after 3 days of  
2 storage in flake ice, although these numbers were significantly lower ( $p < 0.05$ ) than in  
3 the control batch stored for 13 days in flake ice. For both previous treatments, it should  
4 be noted that the microbial concentrations were quite low in all turbot specimens,  
5 according to previous research on flake ice stored turbot (Rodríguez et al., 2003).

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

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

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### 23 **Biochemical analyses**

24 With reference to biochemical analyses (Table 4), TVB-N and FFA values did  
25 not increase as a result of chilled storage time, or produced significant differences

1 (p>0.05) as a result of the kind of ice previously employed. Neither the total volatile  
2 amine formation or lipid hydrolysis development did not indicate differences between  
3 both previous treatments.

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

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

16 According to sensory, microbiological and biochemical quality assessments, it  
17 seems evident that transport in slurry ice system improves the shelf-life of farmed turbot  
18 even when this kind of fish is subsequently transferred to traditional ice and maintained  
19 at a 2°C room temperature in the retail market. Results confirm the practical advantages  
20 for fish industry of using slurry ice as a chilled storage method to inhibit quality loss  
21 during fish processing and retain sensory and nutritional properties. The fact that slurry  
22 ice has also been shown to be an advantageous slaughtering method (Huidobro et al.,  
23 2001) provides an additional reason for employing this binary technology for transport  
24 from fish farms to retail markets.

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**TABLE 1**1  
2**Scale employed for evaluating the sensory quality of chilled turbot**

<b>Attribute</b>	<b>Highest quality (E)</b>	<b>Good quality (A)</b>	<b>Fair quality (B)</b>	<b>Unacceptable quality (C)</b>
Skin	Sharp seaweed and shellfish odour; transparent mucus	Weak seaweed and shellfish odour; milky mucus	Incipiently sour and putrid odour; slightly greyish mucus	Sour and putrid odour; widely opaque mucus
Eyes	Convex; transparent cornea; bright black pupil	Convex and slightly sunken; slightly opalescent cornea; black cloudy pupil	Flat; opalescent cornea; opaque pupil	Concave and milky cornea; by internal organs blurred
Gills	Without odour; brightly red	Without odour; rose coloured	Incipient fishy odour; slightly pale	Intense ammonia odour; grey-yellowish colour
Muscle	Sharp seaweed and shellfish odour; bright-red blood spots	Weak seaweed and shellfish odour; darker red blood spots	Incipiently sour and putrid odour; brown-red blood spots	Sour and putrid odour; brown blood spots

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**TABLE 2**

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

Chilled storage time (days)	Skin				Eyes		Gills				Muscle			
	Odour		Mucus				Odour		Colour		Odour		Bleeding	
	FI	SI	FI	SI	FI	SI	FI	SI	FI	SI	FI	SI	FI	SI
<b>10</b>	A	A	A	E	A	E	A	A	A	A	A	A	A	A
<b>10 (+ 1 day in FI)</b>	A	A	B	A	B	B	A	A	B	A	A	A	A	A
<b>10 (+ 3 days in FI)</b>	B	B	B	A	B	B	B	B	B	A	B	B	B	B
<b>17</b>	B	A	B	A	B	A	B	A	B	A	A	A	A	A
<b>17 (+ 1 day in FI)</b>	C	B	B	A	B	B	C	B	C	A	C	A	B	A
<b>17 (+ 3 days in FI)</b>	C	B	C	B	C	B	C	B	C	B	C	B	C	B

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

9 \*\* Ten and seventeen days in flake ice (FI) and slurry ice (SI) were followed by 0, 1 and 3 days in FI.

**TABLE 3****Quantitative microbial analyses\* of chilled turbot stored in each of the refrigeration strategies evaluated\*\***

Chilled storage time (days)	Aerobes (log CFU g <sup>-1</sup> )		Anaerobes (log CFU g <sup>-1</sup> )		Coliforms (log CFU g <sup>-1</sup> )	
	FI	SI	FI	SI	FI	SI
<b>10</b>	1.58 ± 1.43 b	0.00 ± 0.00 a	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
<b>10 (+ 1 day in FI)</b>	3.50 ± 0.40 b	2.81 ± 0.32 a	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
<b>10 (+ 3 days in FI)</b>	5.76 ± 0.37 b	4.71 ± 0.20 a	1.58 ± 0.57 b	0.43 ± 0.75 a	1.59 ± 0.36 b	0.33 ± 0.58 a
<b>17</b>	4.75 ± 0.77 b	3.16 ± 0.28 a	1.32 ± 0.28 b	0.43 ± 0.75 a	0.33 ± 0.58	0.00 ± 0.00
<b>17 (+ 1 day in FI)</b>	5.03 ± 0.44 b	3.00 ± 0.00 a	3.21 ± 0.54	2.73 ± 0.23	1.75 ± 0.63	1.79 ± 0.28
<b>17 (+ 3 days in FI)</b>	7.11 ± 0.43 b	3.55 ± 0.95 a	3.47 ± 0.32 b	1.56 ± 0.54 a	2.15 ± 0.78 b	1.10 ± 0.17 a

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8 \* Mean values (n = 3) followed by standard deviations are indicated. For each kind of analysis, values followed by different letters are  
9 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 ±  
10 0.00 (coliforms).

11 \*\* 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\*\***

Chilled storage time (days)	TVB-N (mg TVB-N kg <sup>-1</sup> )		TMA-N (mg TMA-N kg <sup>-1</sup> )		K value		FFA (g FFA kg <sup>-1</sup> lipids)		FR	
	FI	SI	FI	SI	FI	SI	FI	SI	FI	SI
<b>10</b>	213.5 ± 33.4	234.5 ± 47.3	0.60 ± 0.05	0.61 ± 0.09	40.7 ± 4.5 b	20.6 ± 3.5 a	13.63 ± 6.66	8.10 ± 5.70	0.80 ± 0.16	0.50 ± 0.34
<b>10</b> (+ 1 day in FI)	221.1 ± 20.3	206.5 ± 33.4	0.68 ± 0.07	0.53 ± 0.08	47.0 ± 5.4 b	32.7 ± 6.4 a	10.10 ± 1.23	5.67 ± 4.76	0.60 ± 0.17	0.67 ± 0.30
<b>10</b> (+ 3 days in FI)	210.0 ± 3.5	225.8 ± 8.8	0.79 ± 0.06	0.66 ± 0.12	57.0 ± 6.9 b	36.0 ± 4.2 a	8.57 ± 1.10	11.73 ± 4.38	0.53 ± 0.12	0.54 ± 0.49
<b>17</b>	196.0 ± 12.6	211.8 ± 15.0	3.47 ± 1.51 b	0.81 ± 0.07 a	60.7 ± 2.0 b	45.0 ± 5.8 a	14.27 ± 5.67	16.10 ± 5.89	1.44 ± 0.50	1.44 ± 0.84
<b>17</b> (+ 1 day in FI)	187.8 ± 9.6	197.2 ± 13.6	2.81 ± 1.13 b	0.77 ± 0.17 a	63.3 ± 1.2 b	42.0 ± 2.8 a	9.87 ± 4.86	11.73 ± 8.06	1.35 ± 0.60	0.71 ± 0.22
<b>17</b> (+ 3 days in FI)	217.0 ± 6.3	222.8 ± 14.0	4.44 ± 3.63	2.82 ± 2.70	60.8 ± 4.1	59.4 ± 2.3	13.00 ± 5.38	9.90 ± 4.59	2.66 ± 1.54	2.08 ± 0.71

\* 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.