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Enhanced quality and safety during on-board chilled storage of fish species captured in the Grand Sole North Atlantic fishing bank

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Abstract

The Grand Sole North Atlantic fishing bank is exploited by several European countries, although time lapsed between catch and destiny arrival can attain 15 days. In the present work, the use of slurry ice (SI) system was investigated for the on-board storage of chilled fish and carried out in parallel to traditional flake icing (FI). Three species (hake, *Merluccius merluccius*; angler, *Lophius piscatorius*; ray, *Raja clavata*) widely present in the mentioned bank were studied. A lower ($p < 0.05$) microbial (aerobes, psychrotrophes, proteolytics) development was observed in fishes subjected to SI system than in their counterpart specimens stored under FI. This correlated with lower ($p < 0.05$) productions of trimethylamine (hake and angler) and total volatile bases (ray) and extended shelf-life for fish species kept under SI conditions. In summary, on-board employment of SI can provide higher quality and safety products to consumer and allow increased commercial values while unloading and sale.
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Keywords: Hake; Angler; Ray; Grand Sole; On-board chilling; Quality; Safety

1. Introduction

Marine species deteriorate rapidly *post mortem*. The degradation process is carried out at first by muscle enzymes and later by microbial enzymes (Olafsdóttir et al., 1997; Whittle, Hardy, & Hobbs, 1990). Unlike other muscle food, fish are usually harvested in remote locations, making the time between the catch and the landing of the fish material much longer than the time between landing and selling (Ward, 1994). As a result of this, the threats of having fish condemned, withdrawn from sale, or sold at low prices at harbour, limit the length of the voyage (Kelman, 1982).

Bacterial growth during storage increases with handling and is due to the direct contact with decks, equipment and boxes (Huss et al., 1974; López-Caballero, Huidobro, Pastor, & Tejada, 2002). In a first step, bacteria present in the gills, gut and skin metabolise low-molecular-weight compounds, producing volatile compounds associated with spoilage. The potential health risks associated with fresh seafood, together with the long distances from local fishing ports, make the need for long-term preservation techniques to be addressed continually (Ashie, Smith, & Simpson, 1996).

In order to slow down the mechanisms involved in quality loss, the fish should be refrigerated immediately after capture. Therefore, several preservation systems such as traditional ice (Nunes, Batista, & Morâo de Campos, 1992), refrigerated seawater (Kraus, 1992), and the addition of chemical preservation agents (Hwang & Regenstein, 1995) have been applied to fish species. Recently, slurry ice (SI) has been reported to be a profitable technique for the preservation of aquatic food products at subzero temperatures (Yamada, Fukusako, & Kawanami, 2002). Its use has been proven to slow down microbial growth, leading

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to significant increases in the shelf life of a broad variety of chilled marine species, such as lean fish (Rodríguez, Barros-Velázquez, Piñeiro, Gallardo, & Aubourg, 2006), fatty fish (Campos, Rodríguez, Losada, Aubourg, & Barros-Velázquez, 2005) and crustaceans (Huidobro, López-Caballero, & Mendes, 2002).

The present work is focused on the catch, on-board storage and commercialisation of three abundant fish species (European hake, *Merluccius merluccius*; angler, also called monkfish, *Lophius piscatorius*; thornback ray, *Raja clavata*) from the Grand Sole North Atlantic fishing bank. This distant bank, exploited by several European countries, is so distant that the time elapsed between the catch and arrival at destination varies from 10 to 15 days. In order to provide consumers with fish of the highest quality and safety, a slurry ice prototype was installed in a fishing vessel, for the on-board refrigeration and storage of the above mentioned species. Sensory, microbiological and chemical analyses on such fish were compared with counterpart batches stored in parallel in flake ice (FI).

2. Materials and methods

2.1. Refrigeration systems

An SI prototype (FLO-ICE, Kinarca S.A.U., Vigo, Spain) was installed in the ship *Patricia-Marta*, based in Vigo fishing harbour (North-western Spain). The composition of the SI binary mixture was 40% ice and 60% water, prepared on-board with filtered sea water (salinity: 3.3%). The temperature of the SI mixture was −1.5 °C. FI was prepared on-board using fresh water with an Icematic F100 Compact device (Castelmac SPA, Castelfranco, Italy); the temperature of the FI was −0.5 °C.

2.2. Fish material, processing and sampling

European hake (*M. merluccius*; length 30–35 cm, weight 170–210 g), angler (*L. piscatorius*; length 37–45 cm, weight 190–230 g) and thornback ray (*R. clavata*; length 40–55 cm, weight 330–370 g) were captured on the Grand Sole North Atlantic fishing bank during a single trip. Hake and angler were gutted immediately after catching, while ray were not. None of the fish species were beheaded. For each fish species, individuals were divided on-board into SI and FI treatments. Individuals were surrounded by either SI or FI at a fish:ice ratio of 1:1, and stored on-board in a refrigerated room at 0–1 °C. Each fish species was captured at four different times during the trip, and at each time, individuals were separated into three groups, which were analysed separately ($n = 3$).

Once the fish specimens were unloaded at Vigo fishing harbour, they were transported to the laboratory and kept in a cold room (0–1 °C) in each type of ice, before analyses were carried out. Sensory, microbiological and chemical analyses were performed after 4, 8, 12 and 16 days (hake and angler) and after 3, 6, 10 and 14 days (ray) of chilled storage. Sensory analysis was carried out on the whole fish, while microbiological and chemical analyses were carried out on the white muscle.

2.3. Sensory analyses

Sensory analysis was conducted by a panel consisting of five experienced judges, according to traditional guidelines concerning fresh and refrigerated fish, adapted to the three species under study (Table 1) (Council Regulation, 1990). Four categories were ranked: highest quality (E), good quality (A), fair quality (B), and unacceptable quality (C). Sensory assessment of the fish included the following parameters: skin and mucus development, external odour, gills and gill cavity, eyes, ventral cavity, consistency and flesh odour.

2.4. Microbiological analyses

Samples of 10 g of fish muscle were dissected aseptically from chilled fish specimens, mixed with 90 ml of 0.1% peptone water (Oxoid Ltd., London, UK), and homogenised in a stomacher (Seward Medical, London, UK) as previously described (Ben-Gigirey, Vieites Baptista de Sousa, Villa, & Barros-Velázquez, 1999). In all cases, serial dilutions from the microbial extracts were prepared in 0.1% peptone water. Total aerobes were investigated by surface innoculation in plate count agar (PCA, Oxoid), after incubation at 30 °C for 72 h. Psychrotrophs were also investigated in PCA but incubation was carried out at 7–8 °C for 10 days. *Enterobacteriaceae* were investigated in crystal violet neutral red bile glucose agar (VRBD agar) (Merck, Darmstadt, Germany) after incubation at 37 °C for 24 h. Microorganisms exhibiting a proteolytic phenotype were investigated in casein-agar medium after incubation at 30 °C for 48 h, as previously described (Ben-Gigirey, Vieites Baptista de Sousa, Villa, & Barros-Velázquez, 2000).

2.5. Chemical analyses

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

Total volatile base-nitrogen (TVB-N) values were measured, as described elsewhere (Aubourg, Sotelo, & Gallardo, 1997). Briefly, fish muscle (10 g) was extracted with 6% perchloric acid and brought up to 50 ml, the TVB-N content being determined following steam distillation of the acid extracts rendered alkaline to pH 13 with 20% NaOH, by titration of the distillate with 10 mM HCl. The results were expressed as mg TVB-N per kg of muscle.

Trimethylamine-nitrogen (TMA-N) values were determined by the picrate method, as previously described (Tozawa, Erokibara, & Amano, 1971). This technique involves the preparation of a 5% trichloroacetic acid extract of fish

Table 1
Scale employed for evaluating the sensory quality of chilled fish

Attribute	Highest quality (E)	Good quality (A)	Fair quality (B)	Unacceptable (C)
Skin and mucus development	Very intense pigmentation; transparent mucus	Milky mucus; insignificant pigmentation losses	Slightly greyish mucus; pigmentation without shine	Widely opaque mucus; important pigmentation losses
External odour	Sharply seaweed and shellfish smell	Weakly seaweed and shellfish smell	Incipiently putrid or ammonia odour	Putrid or ammonia odour
Gills and gill cavity	Brightly red; lamina perfectly separated; without odour	Rose coloured; lamina adhered in groups; without odour	Slightly pale; lamina adhered in groups; incipient fishy odour	Grey-yellowish colour; lamina totally adhered; intense ammonia odour
Eyes	Convex; transparent cornea; bright and black pupil	Convex and slightly sunken; slightly opalescent cornea; black and cloudy pupil	Flat; opalescent cornea; opaque pupil	Concave and milky cornea; Internal organs blurred
Ventral cavity	Brightly white; mauve edge around the fins	Brightly white; red spots around the fins	Brightless and white; presence of a wide number of red or yellow spots	Yellowish or greenish; red spots in the flesh muscle
Consistency	Presence or partial disappearance of *rigor mortis* symptoms	Firm and elastic; pressure signs disappear immediately and completely	Presence of mechanical signs; elasticity notably reduced	Important shape changes due to mechanical factors
Flesh odour	Sharply seaweedy and shellfish smell	Weakly seaweedy and shellfish smell	Incipiently putrid or ammonia odour	Putrid or ammonia odour

muscle (10 g in 25 ml). The results were expressed as mg TMA-N per kg of muscle.

2.6. Statistical analyses

Bacterial counts were transformed into log CFU per g of muscle before undergoing statistical analysis. Data corresponding to both chilling methods were subjected to one-way analysis of variance, to assess significant ($p < 0.05$) differences between treatments. SPSS 11.5 for Windows software (SPSS Inc., Chicago, IL) was also used to explore the statistical significance of the results obtained; this included multiple comparisons by the DMS test. A confidence interval at the 95% level ($p < 0.05$) was considered in all cases.

3. Results and discussion

3.1. Composition analyses

Lipid and water amounts determined were in agreement with those previously reported for lean fish species (Piclet, 1987). Thus, the lipid contents obtained were in the ranges of 3.5–5.3, 3.1–4.2 and 4.7–6.1 (g per kg muscle) for hake, angler, and ray, respectively. Moisture values were in the ranges 783–804, 821–833 and 774–796 (g per kg muscle) for hake, angler and ray, respectively. For each fish species, statistical analysis showed that the differences found in lipid and moisture contents throughout the experiment should be attributed to fish-to-fish variation and not to the chilling system or storage time.

The presence of NaCl in the SI chilling medium led to a progressive increase ($p < 0.05$) of NaCl content in fish white muscle (Table 2). Comparison between fish specimens stored under both icing systems showed higher NaCl concentrations in most cases for hake and ray stored under SI conditions, while angler provided higher values only at advanced storage periods (12–16 days). The range of NaCl contents determined in all three species was similar to those previously reported for other fish species stored in SI (Losada, Piñeiro, Barros-Velázquez, & Aubourg, 2005). However, the NaCl contents resulting from the SI treatment were found to be much lower than those reported for fish muscle refrigerated and stored in sea water (Smith, Hardy, McDonald, & Templeton, 1980) or salted fish (Thorarinsdóttir, Arason, Geirsdóttir, Bogason, & Kristbergsson, 2002).

3.2. Microbiological analyses

The development of *Enterobacteriaceae* in fish muscle gave in all cases values lower than 10 CFU per g of muscle, no significant differences being observed for this parameter between FI and SI batches. Average numbers of enteric bacteria were so low that the contribution of this bacterial group to fish spoilage can be discounted.

With respect to the counts of total aerobes (Fig. 1), a higher ($p < 0.05$) development was obtained for fish specimens stored under FI than for their counterparts kept in SI. For both icing conditions, angler specimens did not exhibit significant differences as a result of storage time, while hake and ray specimens showed an increasing ($p < 0.05$) tendency with storage time. In all cases, aerobe counts reached levels slightly above 10^4 CFU per g, such numbers being considerably below those estimated to be required for the spoilage of fish stored under aerobic conditions (Gram & Huss, 1996).

Values determined for psychotrophic bacteria (Fig. 2) in hake and ray specimens stored in SI were significantly ($p < 0.05$) lower than those determined in the FI batches. This was not the case for angler fish, where no significant differences were observed between both icing conditions.

Table 2
NaCl content (g per kg of muscle)* of fish specimens chilled under different conditions**

Chilled storage time (days)***	Hake		Angler		Ray	
	FI	SI	FI	SI	FI	SI
4 (3)	3.4 (0.1)	3.7 (0.3)	3.9 (0.5)	4.6 (0.2)	3.4 a (0.1)	4.4 b (0.5)
8 (6)	3.5 a (0.4)	4.6 b (0.4)	4.2 (0.5)	4.4 (0.2)	4.3 a (0.5)	5.2 b (0.2)
12 (10)	3.6 a (0.2)	4.7 b (0.3)	4.4 a (0.2)	5.2 b (0.3)	4.3 a (0.5)	5.8 b (0.4)
16 (14)	3.9 a (0.2)	4.9 b (0.1)	4.5 a (0.1)	5.5 b (0.2)	4.4 a (0.2)	6.7 b (0.1)

* For each species, mean values ($n = 3$) followed by different letters indicate significant differences ($p < 0.05$) between both chilling conditions at the storage time expressed; standard deviations are in brackets.
** Chilling conditions: FI (flake ice) and SI (slurry ice).
*** Chilled storage times in brackets correspond to ray fish experiment.

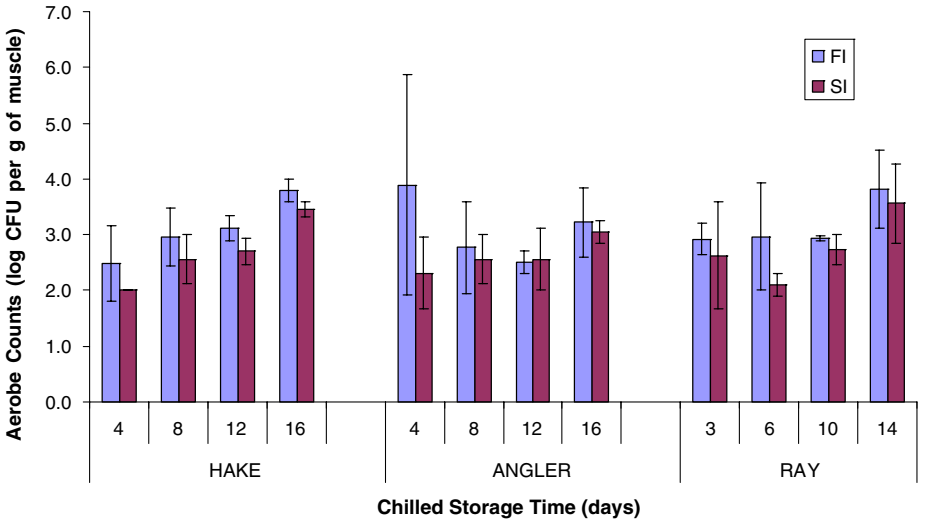

Fig. 1. Total aerobe assessment* in fish (hake, angler, and ray) muscle during storage under different chilling conditions**. *Mean values of three independent determinations ($n = 3$) are presented; bars denote standard deviations. **Chilling conditions: FI (flake ice) and SI (slurry ice).

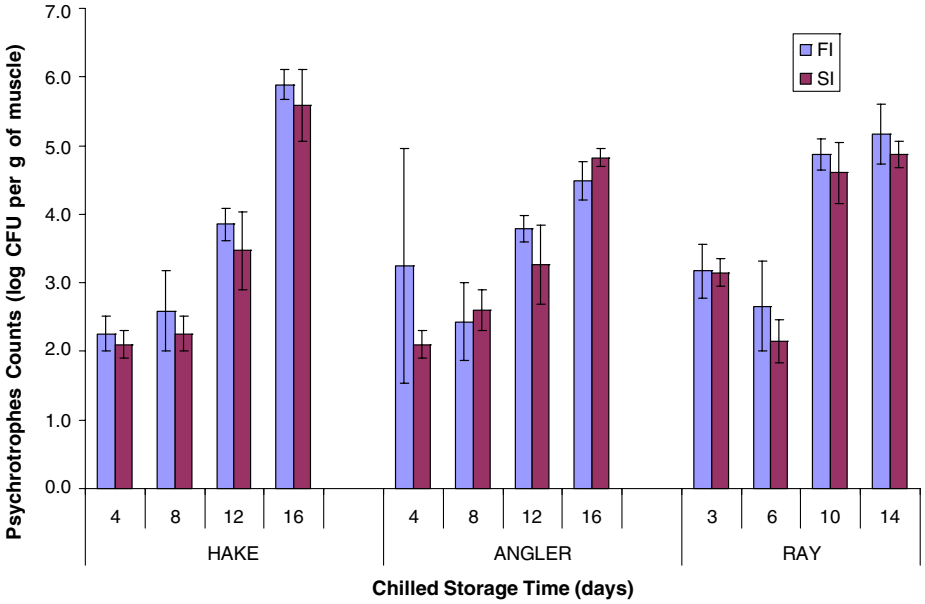

Fig. 2. Psychrotrophic bacteria assessment* in fish (hake, angler, and ray) muscle during storage under different chilling conditions**. *Mean values of three independent determinations ($n = 3$) are presented; bars denote standard deviations. **Chilling conditions as expressed in Fig. 1.

In all cases, an increase ($p < 0.05$) with time was observed, so that counts slightly below 10^6 were obtained for hake and around 10^5 CFU per g for angler and ray.

For all three fish species studied, the proteolytic bacteria (Fig. 3) showed significantly ($p < 0.05$) lower values for specimens stored under SI than for their counterparts kept under FI conditions. In all cases, only slight increases with time of storage were observed.

3.3. Chemical analyses of microbial activity

Chemical assessment of microbial activity was carried out by means of TVB-N and TMA-N analyses.

For hake and angler, the TVB-N value (Table 3) showed a higher mean value in most cases for both species stored under FI conditions than in their counterparts treated under the SI system; however, this index did not increase with storage time in any case. A lack of formation of TVB-N compounds had also been observed during the chilling of hake in flake ice (Baixas-Nogueras, Bover-Cid, Veciana-Nogués, & Vidal-Carou, 2002), although no previous information concerning angler fish was available prior to our study.

With respect to ray, an increasing tendency ($p < 0.05$) of the TVB-N content with storage time (Table 3) was observed for both icing conditions. Thus, a sharp increase ($p < 0.05$) was observed at day 10, as a result of the end of the microbial lag phase, this increase being higher for fish stored under FI conditions. As a result, higher total volatile amine formation was observed in ray fish kept under FI conditions.

Marine elasmobranches such as ray produce and retain within their bodies large amounts of urea (Finne, 1992; Read, 1968). The high TVB-N content found for ray in the present study can be explained as a consequence of microbial decomposition of urea into ammonia (Finne, 1992; Vyncke, 1978). Hence the TVB-N values were higher (Table 3) for ray fish at all times studied.

Changes produced in the TMA-N value (Table 4) throughout the storage time showed a different tendency in ray from the other two fish species under study. For hake and angler, a significant ($p < 0.05$) formation of trimethylamine (TMA) was observed with storage time in both chilling systems, with higher formation ($p < 0.05$) in angler than in hake. Comparison between both icing conditions showed higher average values in most cases for hake and angler individuals stored under FI conditions than in their counterparts subjected to SI. A sharp TMA formation ($p < 0.05$) was observed at day 16 for these two species stored under FI conditions, corresponding to the end of the lag phase. Results on TMA formation in hake are in agreement with previous research (Baixas-Nogueras et al., 2002; Ruiz-Capillas & Moral, 2001), while no previous data are available concerning chilled angler.

For hake and angler, TMA-N assessment has shown to be a more accurate index than TVB-N value. This can be explained by the fact that the latter quantifies a wide range of basic volatile compounds (ammonia, methylamine, dimethylamine, trimethylamine, etc.) produced by different breakdown pathways, while TMA-N assessment accounts only for trimethylamine oxide breakdown.

In contrast, when ray fish specimens are considered (Table 4), significant differences in TMA-N values were not observed between specimens kept under both icing systems. It is well known that TMA is produced during the chilled storage of fish, as a result of the bacterial breakdown of trimethylamine oxide (TMAO) (Finne, 1992). In this sense, TMA-N values in ray were markedly higher than those obtained in the two other species; this result agrees with the higher TMAO amounts found in cartilaginous fishes than in other kinds of fish (Finne, 1992; Read, 1968).

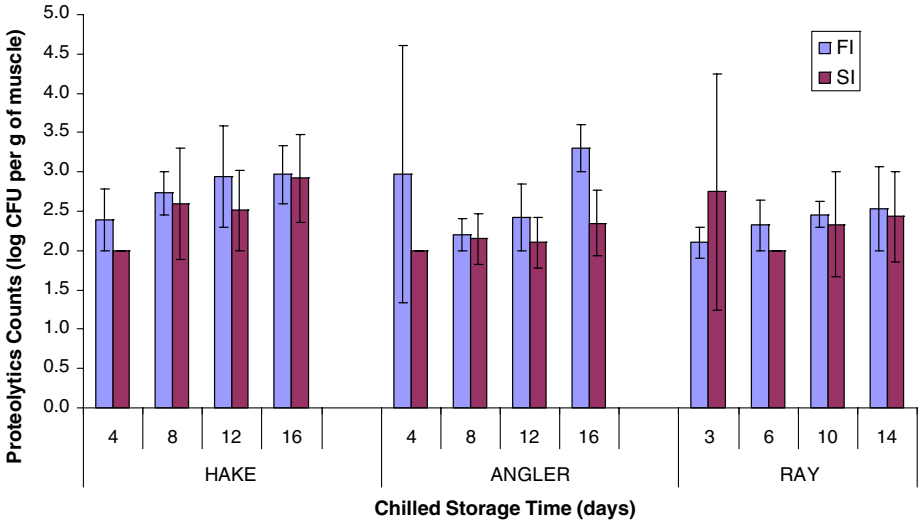

Fig. 3. Proteolytic bacteria assessment* in fish (hake, angler, and ray) muscle during storage under different chilling conditions**. *Mean values of three independent determinations ($n = 3$) are presented; bars denote standard deviations. **Chilling conditions as expressed in Fig. 1.

Table 3
Changes in total volatile base-nitrogen (TVB-N) content* (mg TVB-N per kg of muscle) of chilled fish**

Chilled storage time (days)***	Hake		Angler		Ray	
	FI	SI	FI	SI	FI	SI
4 (3)	238.2 (22.5)	207.7 (22.5)	182.7 (16.0)	178.3 (13.6)	341.0 (10.2)	333.8 (16.4)
8 (6)	230.5 (50.0)	198.3 (19.2)	183.3 (27.4)	193.3 (20.9)	352.6 (12.5)	337.1 (3.8)
12 (10)	228.3 (11.7)	183.3 (39.8)	186.6 (10.1)	177.7 (6.3)	401.0 b (17.1)	377.1 a (6.7)
16 (14)	206.6 (10.1)	199.9 (5.0)	190.5 (13.4)	169.4 (11.7)	668.6 b (67.6)	439.3 a (41.8)

* For each species, mean values ($n = 3$) followed by different letters indicate significant differences ($p < 0.05$) between both chilling conditions at the storage time expressed; standard deviations are in brackets.
** Chilling conditions as expressed in Table 2.
*** Chilled storage times in brackets correspond to ray fish experiment.

Table 4
Changes in trimethylamine-nitrogen (TMA-N) content* (mg TMA-N per kg of muscle) of chilled fish**

Chilled storage time (days)***	Hake		Angler		Ray	
	FI	SI	FI	SI	FI	SI
4 (3)	1.3 (0.6)	0.8 (0.1)	4.3 (0.8)	3.3 (0.3)	40.0 (4.1)	42.8 (6.6)
8 (6)	1.0 (0.2)	0.9 (0.1)	4.6 (1.7)	4.8 (1.9)	32.6 (12.8)	40.9 (6.7)
12 (10)	1.9 (0.5)	1.6 (0.2)	5.4 b (0.4)	4.6 a (1.7)	26.1 (8.8)	36.5 (8.3)
16 (14)	4.8 b (1.4)	1.9 a (0.3)	12.3 b (1.9)	5.3 a (1.9)	28.9 (4.1)	25.2 (4.1)

* For each species, mean values ($n = 3$) followed by different letters indicate significant differences ($p < 0.05$) between both chilling conditions at the storage time expressed; standard deviations are in brackets.
** Chilling conditions as expressed in Table 2.
*** Chilled storage times in brackets correspond to ray fish experiment.

A most remarkable result in the present ray fish study was the decreasing tendency of TMA-N value with storage time ($p < 0.05$) for both icing conditions. It has been proposed that a physiological function of TMAO in elasmobranch species is the elimination of waste ammonia, which otherwise would reach toxic concentrations; this elimination is accomplished through transmethylation and oxidation (Baldwin, 1957; Walsh & Smith, 2001). In the present study, the increasing TVB-N value observed in ray as storage time progressed clearly indicated increasing ammonia formation. As a result of its abovementioned function, TMAO content would be gradually lost with increasing decomposition of the ray specimens. Consequently, a TMA formation decrease would occur as ray decomposition increases. The experimental data provided in this study strongly support this theory. Thus, the role of TMAO in ammonia elimination would limit the amount of TMAO available as a substrate for bacterial activity, limiting TMA formation at advanced storage times, as ray spoilage progressed.

3.4. Sensory analysis

Hake and angler specimens maintained good quality (categories E and A) up to day 8 when stored under SI conditions (Table 5); after this time, sensory quality decreased and on day 16 the hake specimens stored in SI were no longer acceptable, while angler was still acceptable. Ray fish specimens exhibited good quality up to day 6, and were still acceptable at the end of the study (day 14). The parameters that gave the worst scores were, consistency and gill cavity (hake), eyes (angler) and external ammonia odour and consistency (ray).

In contrast, specimens stored in flake ice (Table 5) maintained good quality only until day 4 (hake and angler) and day 3 (ray). After these times, sensory quality decreased and the batches exhibited unacceptable quality on days 12 (hake), 16 (angler) and 14 (ray). In the FI batches, the limiting factors were consistency (hake), ventral cavity (angler), and external ammonia odour (ray).

The sensory acceptance decrease found in hake and angler over storage time corresponded with an increase in chemical (TMA-N value) and most microbiological (aerobe, psychrotrophe, and proteolytic counts) parameters. For ray fish, the sensory quality loss corresponds with an increase in the TVB-N value and the microbiological

Table 5
Sensory assessment[a] of fish species stored under different chilling conditions[b]

Chilled storage time (days)[c]	Hake		Angler		Ray	
	FI	SI	FI	SI	FI	SI
4 (3)	A	A	A	A	A	A
8 (6)	B	A	B	A	B	A
12 (10)	C	B	B	B	B	B
16 (14)	C	C	C	B	C	B

[a] Freshness categories as expressed in Table 1.
[b] Chilling conditions as expressed in Table 2.
[c] Chilled storage times in brackets correspond to ray fish experiment.

parameters. The time of sensory quality rejection in angler and ray stored under FI conditions corresponded with a great increase in TMA-N and TVB-N values, respectively. Previous research on chilled European hake (Ruiz-Capillas & Moral, 2001; Baixas-Nogueras et al., 2002) observed longer shelf-life times (20–25 days). In the present work, the shelf-life was lower; this is explained by the relative small size of the hake specimens examined in our study.

4. Concluding remarks

According to microbial, chemical, and sensory analyses, the use of SI instead of FI as a chilling system caused a partial inhibition of microbial activity, resulting in better quality and an extended shelf life, for the fish specimens investigated. The results presented in this work allow us to conclude that the application of SI is advisable for the three species studied. Fish specimens subjected to such conditions would exhibit better quality during unloading, resulting in increased commercial values during their sale.

In accordance with their different chemical composition (urea and TMAO contents), different damage mechanisms were observed in ray, as compared to hake and angler. To our knowledge, the present study provides the first report about the quality loss in angler specimens during their chilled storage.

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References

Ashie, I., Smith, J., & Simpson, B. (1996). Spoilage and shelf-life extension of fresh fish and shellfish. *Critical Reviews in Food Science and Nutrition, 36*, 87–121.

Aubourg, S., Sotelo, C., & Gallardo, J. (1997). Quality assessment of sardines during storage by measurement of fluorescent compounds. *Journal of Food Science, 62*, 295–299.

Baixas-Nogueras, S., Bover-Cid, S., Veciana-Nogués, M., & Vidal-Carou, M. (2002). Chemical and sensory changes in Mediterranean hake (*Merluccius merluccius*) under refrigeration (6–8 °C) and stored in ice. *Journal of Agricultural and Food Chemistry, 50*, 6504–6510.

Baldwin, E. (1957). *Dynamic aspects of Biochemistry* (3rd ed.). London (UK) and New York (USA): Cambridge University Press.

Ben-Gigirey, B., Vieites Baptista de Sousa, J., Villa, T., & Barros-Velázquez, J. (1999). Histamine and cadaverine production by bacteria isolated from fresh and frozen albacore (*Thunnus alalunga*). *Journal of Food Protection, 62*, 933–939.

Ben-Gigirey, B., Vieites Baptista de Sousa, J., Villa, T., & Barros-Velázquez, J. (2000). Characterization of biogenic amine-producing *Stenotrophomonas maltophilia* strains isolated from white muscle of fresh and frozen albacore tuna. *International Journal of Food Microbiology, 57*, 19–31.

Campos, C., Rodríguez, Ó., Losada, V., Aubourg, S., & Barros-Velázquez, J. (2005). Effects of storage in ozonised slurry ice on the sensory and microbial quality of sardine (*Sardina pilchardus*). *International Journal of Food Microbiology, 103*, 121–130.

Council Regulation. (1990). *Official Journal of the European Communities, C84*(19 February), 69.

Finne, G. (1992). Non-protein nitrogen compounds in fish and shellfish. In G. Flick & R. Martin (Eds.), *Advances in Seafood Biochemistry* (pp. 393–401). Lancaster, Pennsylvania (USA): Technomic Publishing Company, Inc.

Gram, L., & Huss, H. (1996). Microbiological spoilage of fish and fish products. *International Journal of Food Microbiology, 33*, 121–137.

Helrich, K. (Ed.). (1990). *Official methods of analysis of the Association of Official Analytical Chemistry* (15th ed., pp. 870). Arlington, Virginia, USA: Association of Official Analytical Chemists', Inc.

Huidobro, A., López-Caballero, M., & Mendes, R. (2002). Onboard processing of deepwater pink shrimp (*Parapenaeus longirostris*) with liquid ice: Effect on quality. *European Food Research Technology, 214*, 469–475.

Huss, H., Dalsgaard, D., Hansen, L., Ladefoged, H., Pedersen, A., & Zitan, L. (1974). The influence of hygiene in catch handling on the storage life of iced cod and plaire. *Journal of Food Technology, 9*, 213–221.

Hwang, K., & Regenstein, J. (1995). Hydrolysis and oxidation of mackerel (*Scomber scombrus*) mince lipids with NaOCl and NaF treatments. *Journal of Aquatic Food Production and Technology, 4*, 19–30.

Kelman, J. (1982). Handling wet fish at sea. In A. Aitken, I. Mackie, J. Merritt, & M. Windsor (Eds.), *Fish: Handling and Processing* (pp. 28–41). Edinburgh, Scotland, UK: Ministry of Agriculture, Fisheries and Food.

Kraus, L. (1992). Refrigerated sea water treatment of herring and mackerel for human consumption. In J. Burt, R. Hardy, & K. Whittle (Eds.), *Pelagic fish. The resource and its exploitation* (pp. 73–81). Aberdeen, Scotland, UK: Fishing News Books.

López-Caballero, M., Huidobro, A., Pastor, A., & Tejada, M. (2002). Microflora of gilthead seabream (*Sparus aurata*) stored in ice. Effect of washing. *European Food Research and Technology, 215*, 396–400.

Losada, V., Piñeiro, C., Barros-Velázquez, J., & Aubourg, S. (2005). Inhibition of chemical changes related to freshness loss during storage of horse mackerel (*Trachurus trachurus*) in slurry ice. *Food Chemistry, 93*, 619–625.

Nunes, M., Batista, I., & Morâo de Campos, R. (1992). Physical, chemical and sensory analysis of sardine (*Sardina pilchardus*) stored in ice. *Journal of the Science of Food and Agriculture, 59*, 37–43.

Olafsdóttir, G., Martinsdóttir, E., Oehlenschläger, J., Dalgaard, P., Jensen, B., Undeland, I., et al. (1997). Methods to evaluate fish freshness in research and industry. *Trends in Food Science and Technology, 8*, 258–265.

Piclet, G. (1987). Le poisson aliment. Composition et intérêt nutritionnel. *Cahiers de Nutrition et Diététique, XXII*, 317–335.

Read, L. (1968). Urea and trimethylamine oxide levels in elasmobranch embryos. *Biological Bulletin of the Marine Biological Laboratory, 135*, 537–545.

Rodríguez, Ó., Barros-Velázquez, J., Piñeiro, C., Gallardo, J., & Aubourg, S. (2006). Effects of storage in slurry ice on the microbial, chemical and sensory quality and on the shelf life of farmed turbot (*Psetta maxima*). *Food Chemistry, 95*, 270–278.

Ruiz-Capillas, C., & Moral, A. (2001). Correlation between biochemical and sensory quality indices in hake stored in ice. *Food Research International, 34*, 441–447.

Smith, J., Hardy, R., McDonald, I., & Templeton, J. (1980). The storage of herring (*Clupea harengus*) in ice, refrigerated sea water and at ambient temperature. Chemical and sensory assessment. *Journal of the Science of Food and Agriculture, 31*, 375–385.

Thorarinsdóttir, K., Arason, S., Geirsdóttir, M., Bogason, S., & Kristbergsson, K. (2002). Changes in myofibrillar proteins during processing of salted cod (*Gadus morhua*) as determined by electrophoresis and differential scanning calorimetry. *Food Chemistry, 77*, 377–385.

Tozawa, H., Erokibara, K., & Amano, K. (1971). Proposed modification of Dyer's method for trimethylamine determination in codfish. In R. Kreuzer (Ed.), *Fish Inspection and Quality Control* (pp. 187–190). London, UK: Fishing News Books Ltd.

Vyncke, W. (1978). Influence of sodium tripolyphosphate and citric acid on the shelf life of thornback ray (*Raja cl*avata). *Zeitschrift für Lebensmittel-Untersuchung und -Forschung A, 166*, 284–286.

Walsh, P., & Smith, C. (2001). Urea transport. In P. Wright & P. Anderson (Eds.), *Nitrogen excretion* (pp. 279–307). San Diego, California, USA: Academic Press.

Ward, D. (1994). Microbiological quality of fishery products. In A. Martin (Ed.), *Fisheries: Processing* (pp. 1–17). London, UK: Chapman and Hall.

Whittle, K., Hardy, R., & Hobbs, G. (1990). Chilled fish and fishery products. In T. Gormley (Ed.), *Chilled foods. The state of the art* (pp. 87–116). USA: Elsevier Applied Science.

Yamada, M., Fukusako, S., & Kawanami, T. (2002). Performance analysis on the liquid-ice thermal storage system for optimum operation. *International Journal of Refrigeration, 25*, 276–277.