1 **Running title:** Enhanced shelf-life of hake stored in slurry ice

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4 **Title:** Enhanced shelf-life of **chilled** European hake (*Merluccius*
5 *merluccius*) stored in slurry ice as determined by sensory **analysis** and
6 microbiological **activity assessment**

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Abstract

Slurry ice, a biphasic system consisting of small spherical ice crystals surrounded by seawater at subzero temperature, was evaluated as a new **chilled** storage method for European hake (*Merluccius merluccius*), a gadoid fish species of remarkable commercial interest, and compared with a control batch stored for 19 days in **traditional** flake ice. The results obtained in the sensory analysis indicated a significant extension of shelf-life, from five days (flake ice batch) to 12 days (slurry ice batch), this correlating well with the observed microbial **activity**. Initial **total volatile base-nitrogen** concentrations of 22.69 mg/100 g muscle at day 0 were maintained in the slurry ice batch along storage, but rose to 47.42 mg/100 g muscle in the flake ice batch after 19 days of storage. Significant differences in the trimethylamine concentrations after 19 days of storage were also observed between the slurry ice batch (3.97 mg/100 g muscle) and flake ice batch (11.54 mg/100 g muscle). Significantly lower counts of total aerobes and proteolytic bacteria were also attained in hake muscle stored in slurry ice, microbial numbers reaching differences above 2 or 3 log units, respectively, after 19 days of storage. The slurry ice batch exhibited a significantly lower increase in the pH value (0.2 units), as compared to the flake ice batch (1.1 units), this also indicating a better control of alkalising microflora. According to the parameters assessed, storage of European hake in slurry ice extended its shelf-life and allowed a better maintenance of sensory and microbiological quality.

Keywords: Slurry ice; Hake; Shelf-life; Sensory quality; Microbiological quality; Chilling; Storage

Hake; chilled storage; slurry ice; Shelf-life; Sensory quality; Microbiological quality

Mencionar primero el pH, después los datos microbiológicos y finalmente las aminos. Mencionar diferencias a nivel de menos días que 19, ya que 19 está muy lejos del tiempo de vida útil, incluso de HL. Mencionar diferencias del día 12 o menos si posible.

1. Introduction

Aquatic food products easily deteriorate *post-mortem* as a consequence of a variety of biochemical and microbial breakdown mechanisms, although the rate of quality loss directly depends on the nature of fish species and on the handling and storage conditions (Whittle, Hardy & Hobbs, 1990; Olafsdóttir et al., 1997). To slow down the mechanisms involved in the quality loss, refrigeration of the fish material immediately after the catch is mandatory. Aquatic food products have traditionally been chilled and stored in conventional flake ice (Nunes, Batista & Morão de Campos, 1992), refrigerated seawater (Kraus, 1992) or subjected to the addition of chemical preservation agents (Ponce de León, Inoue & Shinano, 1993; Hwang & Regenstein, 1995).

Recently, slurry ice, also known as fluid ice, slush ice, liquid ice or flow ice, has been reported as a promising technique for the preservation of aquatic food products in an ice-water suspension at a subzero temperature. Indeed, a review of chilling technologies applied to fisheries defends the use of systems based on ice slurries for the refrigeration of aquatic food products (Graham, Johnston & Nicholson, 1993). Two relevant characteristics of slurry ice are its faster chilling rate, consequence of its higher heat-exchange capacity, and the reduced physical damage caused to aquatic food products by its microscopic spherical particles, as compared with flake ice. Complete coverage of the fish surface by the slurry ice mixture also affords a better protection of the fish material with respect to oxidation and dehydration events. The versatility of the slurry ice technique should also be highlighted. Thus, slurry ice can be pumped, this improving its hygienic handling, and may be combined with other agents such as the cases of ozone, to achieve an antiseptic surface effect, and melanosis inhibitors, to prevent browning reactions in shellfish (Huidobro, López-Caballero & Mendes, 2002).

Although the theoretical advantages of slurry ice are well known, few empirical data reporting the potential practical advantages derived from the use of slurry ice for the storage of marine species are available. According to the literature, the original approaches to the use of slurry ice technologies with aquatic food products date back to the pioneer report by Chapman (1990), who reported a better maintenance of quality of finfish stored in slurry ice on-board, as compared with other chilling technologies. Similar good results were obtained with slurry ice used for the on-board storage of albacore (Price, Melvin & Bell, 1991). Harada (1991) also highlighted the advantages of slurry ice systems as a pre-cooling method. More recently, Martinsdóttir et al. (2002) applied a slurry ice system for the storage of seabass, a warm water fish species, no significant differences in the spoilage rate being observed between the flake ice batch and the slurry ice batch.

Slurry ice has also been reported to be a good slaughter method to sacrifice and store farmed seabream (Huidobro, Mendes & Nunes, 2001), although such specimens stored in slurry ice exhibited cloudy eyes, a result that may be regarded as negative for the commercialisation of this fish species. Practical advantages of slurry ice systems for shellfish storage were also reported for Australian prawns (Chinivasagam et al., 1998), and, more recently for shrimp stored on-board (Huidobro, López-Caballero & Mendes, 2002).

In Spain and in other European countries as well, the gadoid fish represent an important percentage of the overall fish consumption. Inside this group of species, hake is a fish species of great demand in the Spanish market (Piñeiro et al., 1998, 2001, 2003a; Ruíz-Capillas & Moral, 2001). Thus, from the commercial point of view, some members of the genus *Merluccius* deserve a relevant appreciation as fresh fish because

1 of their excellent organoleptic features. Among them, *Merluccius merluccius*,
2 commonly known as European hake, is the most appreciated species and deserves the
3 highest commercial value in Spain, being preferred to other species of the genus
4 *Merluccius* and to other gadoids. The fishing of European hake in distant fishing banks
5 usually implies that the time elapsed between the caught and the arrival to the port may
6 oscillate from three to ten days, a fact that underlines the need of optimising the
7 refrigeration parameters to provide the consumer with fish of the highest quality
8 possible. Accordingly, in the present work we evaluate the effect of chilling European
9 hake in slurry ice on its shelf-life, sensory, and microbiological quality. The results
10 were compared with those determined in parallel in a batch stored in flake ice during 19
11 days.

2. Materials and methods

2.1. Slurry ice system used

In this work, a slurry ice prototype (FLO-ICE, Kinarca S.A.U., Vigo, Spain) was used. The composition of the slurry ice binary mixture was 40% ice and 60% water, prepared from filtered seawater (salinity: 3.3%). The temperature of the slurry ice mixture was -1.5°C. Flake ice was prepared with an Icematic F100 Compact device (CASTELMAC SPA, Castelfranco, Italy). The fish specimens were surrounded by slurry or flake ice at a fish:ice ratio of 1:1, and stored for up to 19 (flake ice batch) or 22 (slurry ice batch) days in a refrigerated room at 2°C. When required, the flake ice and the slurry ice mixture were renewed.

2.2. Fish material, processing and sampling

European hake (*Merluccius merluccius*) specimens were caught in the day and kept in ice as they arrived at our laboratory. The fish specimens were not headed nor gutted. The length of the specimens was in the range of 25–30 cm and the weight was 150–200 g. Fish species were chilled in slurry ice and in traditional flake ice. For each chilled treatment, three different batches were used and studied separately along the whole experimental period. Samples were taken from each batch on days 0, 2, 5, 8, 12, 15, 19, and, in the case of slurry ice, also at day 22. Once whole fish had been subjected to sensory analyses, the white muscle was separated and used for microbiological and chemical analyses. All analyses were performed in triplicate.

2.3. *Sensory analyses*

Sensory analysis was conducted by a sensory panel consisting of five experienced judges, according to traditional guidelines (Table 1) concerning fresh and refrigerated fish (DOCE, 1989; Rodríguez et al., 2003a). 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. Meior eliminar.

2.4. *Microbiological analyses*

Samples of 25 g of fish muscle were dissected aseptically from chilled hake specimens, mixed with 225 ml of 0.1% peptone water, and homogenised in a stomacher (Seward Medical, London, UK), as previously described (Ben-Gigirey et al., 1998, 1999). In all cases, serial dilutions from the microbial extracts were prepared in 0.1% peptone water. Total aerobes were investigated in plate count agar (PCA, Oxoid Ltd., London, UK) after incubation at 31°C for 72 h. Anaerobes were investigated in the same manner, except that an anaerobic atmosphere kit (Oxoid) was placed together with the plates inside the anaerobiosis jar. Lactose-fermenting *Enterobacteriaceae* (coliforms) were investigated in Violet Red Bile Agar (VRBA medium, Merck, Darmstadt, Germany) after incubation at 30°C±1°C for 24±2 h, as recommended by the manufacturer (Merck Microbiology Manual, 2002). Microorganisms exhibiting a proteolytic phenotype were investigated in casein-agar medium (30°C/48 h) (Phaff et al., 1994), as previously described (Ben-Gigirey et al., 2000).

2.5. Chemical analyses

The evolution of pH values in hake muscle along storage time was determined by means of a 6-mm diameter insertion electrode (Crison, Barcelona, Spain).

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, after 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/100 g muscle.

Trimethylamine-nitrogen (TMA-N) values were determined by the picrate method, as previously described (Tozawa, Erokibara & Amano, 1971). This involves the preparation of a 5% trichloroacetic acid extract of fish muscle (10 g /25 ml). Results were expressed as mg TMA-N/100 g muscle.

2.6. Statistical analyses

Bacterial counts were transformed into log CFU/g before subjecting to statistical analyses. The SPSS 11.5 for Windows software (SPSS Inc., Chicago, IL) was used to explore the statistical significance of the results obtained, this including multivariate contrasts and multiple comparisons by the Tukey test. A confidence interval at the 95% level ($p < 0.05$) was considered in all cases.

3. Results and discussion

3.1. Sensory analyses

According to the results of the sensory analyses, the hake specimens stored in slurry ice maintained good quality, being classified in the E or A categories, up to day 12 (Table 2). After this time, quality decreased, and on day 15 and onwards the hake specimens stored in slurry ice were no longer acceptable. The main aspect related to quality loss in the slurry ice batch corresponded to the appearance of the eyes. However, and unlike the report on seabream stored in slurry ice (Huidobro, Mendes & Nunes, 2001), such defect was observed in hake only after 15 days of storage. The parameters that deserved the best scores in the hake stored in slurry ice were the gills and the flesh odor, which merited acceptable rates even after 22 days of storage.

In contrast with these results, the good sensory quality, E and A categories, of hake specimens stored in flake ice was only maintained up to day **two**, such hake specimens being rejected on day eight (Table 2). In the case of turbot stored in flake ice, the gill color and odor resulted to be the limiting factors of acceptability (Piñeiro et al., 2003b). The shelf-life of hake in flake ice reported in this work is in agreement with the results obtained by Ruiz-Capillas and Moral (2001). Accordingly, storage of hake in slurry ice allowed a significant extension of its shelf-life.

3.2. Microbiological analyses

One-way ANOVA was initially carried out considering aerobes, anaerobes, coliforms, and proteolytic bacteria as dependent variables, and time as the factor. *Post-hoc* analyses were selected and the Tukey test was performed. Figs. 1 and 2 and Table 3 show the most relevant results concerning microbial growth in hake stored in either slurry ice or flake ice. Thus, statistically-significant ($p < 0.05$) differences were observed between both batches for aerobes and proteolytic bacteria. In the case of the counts of total aerobes, the average difference between batches was 1.13 log units at day 12, while such difference rose to 1.35 and 2.33 log units after 15 and 19 days of storage, respectively (Fig. 1). In global terms, the average difference determined for both batches until day 19, this being the last sampling time for flake ice, was 1.11 log units. Although the total bacterial counts did not reach levels of 10^8 CFU/g, which are considered to be required for the spoilage of fish stored aerobically (Gram & Huss, 1996), storage of hake in slurry evidenced a significant slow down of bacterial growth. These results are in agreement with the significantly lower counts determined for turbot (Rodríguez et al., 2003b), horse mackerel (Rodríguez et al., 2003c), and shrimp (Huidobro, López-Caballero & Mendes, 2002) stored in slurry ice, as compared with counterpart batches stored in flake ice. Accordingly, storage of hake in slurry ice implied, as in the above-cited fish species, a significantly slower growth of the total aerobes with respect to storage in traditional flake ice.

It is widely accepted that microbial metabolites such as peptides or amino acids, derived from protein hydrolysis, contribute significantly to undesirable sensory changes in seafood products. Thus, such undesirable modifications in the odor, texture and appearance lead to the spoilage of seafood products (Shewan, 1977; Makarios-Laham & Lee, 1993; Rodríguez et al., 2003a). Accordingly, the comparative evolution of proteolytic bacteria in both batches was investigated. As it can be observed in Fig. 2, statistically-significant ($p < 0.05$) lower counts of proteolytic bacteria were observed in the slurry ice batch than in the flake ice batch. The counts of proteolytic bacteria in hake

muscle stored in flake ice reached levels above 10^5 CFU/g at day 12, while the slurry ice batch only reached counts of 10^4 CFU/g even at day 22. Significant differences of 1.14 and 1.68 log units were determined in the counts of proteolytic bacteria between batches, at days 8 and 12 of storage, respectively. As in the case of aerobes, this difference increased with the time of storage, and reached values even above 3 log units after 15 days of storage. In global terms, the average difference between the counts of proteolytic bacteria determined for both batches was 1.75 log units, this clearly indicating a significant decrease in the growth of such bacterial group in hake muscle stored in slurry ice. These results confirm previous works that indicated a significant slower growth of bacteria exhibiting a proteolytic phenotype in turbot (Rodríguez et al., 2003b), and horse mackerel (Rodríguez et al., 2003c) stored in slurry ice, in comparison to storage in conventional flake ice.

The average counts of anaerobes in hake muscle stored in slurry ice were 1.54 log CFU/g, this being lower than those determined in the flake ice batch: 2.14 log CFU/g (Table 3). However, such difference was not found to be statistically-significant at the $p < 0.05$ level. Moreover, the average counts obtained for anaerobes in each batch neither exhibited statistically-significant differences with respect to the initial counts at day 0 (2.28 log CFU/g), this indicating: (i) a remarkably good initial quality of the fish material, and (ii) a very limited growth of anaerobes during chilled storage regardless the ice system considered.

With respect to the development of coliforms in hake muscle stored in either slurry ice or flake ice, no significant ($p < 0.05$) differences were observed between both batches (Table 3). Again, the average counts of coliforms were so low (below 1 log CFU/g) in both batches, that no statistically-significant ($p < 0.05$) difference was observed between both batches, neither with respect to the initial counts at day 0, this also confirming the good initial quality of the fish material studied in this work.

Finally, it should also be stressed that the results obtained in the microbiological analyses, especially those obtained for the total aerobes and proteolytic bacteria, correlated well with the differences observed in the sensory studies, as also described in previous reports for turbot (Rodríguez et al., 2003b), horse mackerel (Rodríguez et al., 2003c), and shrimp (Huidobro, López-Caballero & Mendes, 2002), thus confirming the better maintenance of quality in hake stored in slurry ice.

3.3. Chemical analyses

Increases in the pH value indicate the accumulation of alkaline compounds, such as ammonia and TMA, mainly derived from the microbial action (Hebard, Flick & Martin, 1982). In the case of hake, pH values higher than 7 may limit its consumption (Moral, 1987, Ruíz-Capillas & Moral, 2001). In our work, statistically-significant differences were determined for pH between both batches (Fig. 3). Thus, the initial pH value of 6.67 of hake muscle increased slightly during storage in slurry ice, reaching a value of 6.83 at the end of the storage period (22 days). Thus, in the slurry ice batch, the pH value was always below 7. Similar slight increases had also been reported for turbot (Rodríguez et al., 2003b; Piñeiro et al., 2003b) and horse mackerel (Rodríguez et al., 2003c; Losada et al., 2003) stored in slurry ice. On contrast, the pH value of hake muscle steadily increased in the batch stored in flake ice from the initial 6.67 to values of 6.98 and 7.71 after five and 19 days of storage, respectively. In our work, the highest increase in the pH value was observed in the flake ice batch after 12 days of storage, this coinciding with the highest increases in the counts of total aerobes and proteolytic bacteria in such batch. This result suggests that the better microbial control achieved in

the slurry ice batch would have allowed a less intense development of alkalising microflora, this leading to a better maintenance of the pH value. Likewise, the important pH increase determined in hake muscle stored in flake ice is in agreement with previous studies carried out on turbot (Rodríguez et al., 2003a) and horse mackerel (Rodríguez et al., 2003c; Losada et al., 2003) at our laboratory, and with other authors that reported steady increases in the pH value for other fish species stored in conventional flake ice (Nunes, Batista & Morão de Campos, 1992; Ruíz-Capillas & Moral, 2001).

The TVB-N content of hake stored in slurry ice showed very slight differences along the storage time (Fig. 4). Thus, the evolution of the TVB-N content in the slurry ice batch showed an almost horizontal profile up to day 19, this being followed by an increasing trend up to the end of the storage period (Fig. 4). The final average TVB-N value for the slurry ice batch was quite low: 26 mg TVB-N/100 g muscle, this indicating a significantly low production of volatile bases in hake muscle stored in slurry ice. As expected statistical analyses confirmed that the storage of hake in slurry ice significantly slowed down TVB-N formation in comparison with storage in flake ice. In the latter batch, TVB-N concentrations exhibited a sharp increase after 12 days of storage –this coinciding with the most remarkable increases in the microbial counts of total aerobes and proteolytic bacteria (Figs. 1 and 2)– and reached final concentrations above 47 mg TVB-N/100 g muscle. Unlike the flake ice batch, hake stored in slurry ice never reached the legal limit of 35 mg/100 g set for TVB-N (Directive 95/149/EEC). Similar important increases in the TVB-N content of hake after 9-12 days of storage in flake ice had previously been reported, this coinciding with the end of the microbial lag phase (Ruíz-Capillas & Moral, 2001; Baixas-Nogueras et al., 2002).

The TMA-N index of hake muscle stored in either flake ice or slurry ice seldom increased in the period elapsed between 0-12 days of storage in flow ice (Fig. 5). After this time, a sharp increase was observed in the flake ice batch up to the end of storage, this leading to a final value above 11.5 mg TMA-N/100 g muscle after 19 days of storage (Fig. 5), this being very close to the legal limit of 12 mg/100 g set for TMA-N (Directive 91/493/EEC). However, other authors have considered a maximum TMA-N level of 5 mg/100 g for hake (Ludorff & Meyer, 1978; Baixas-Nogueras et al., 2002). However, the TMA-N content of hake muscle stored in slurry ice was always below 5 mg/100 g, even after 22 days of storage.

In agreement with these results, other reports concerning hake (Ruíz-Capillas & Moral, 2001; Baixas-Nogueras et al., 2002) and other fish species (Fernández-Salguero & Mackie, 1987; Pérez-Villarreal & Pozo, 1990) had described sharp increases in TMA-N contents after 9-12 days of storage in conventional flake ice. In contrast with these results, the final TMA-N content determined in the slurry ice batch was below 4 mg TMA-N/100 g muscle. As expected, statistical analyses confirmed that the differences found for TMA-N between both batches were significant at the $p<0.05$ level. Accordingly, storage of hake in slurry ice also slowed down TMA-N formation in comparison with hake stored in conventional flake ice.

4. Conclusions

In sum, the storage of European hake in slurry ice at subzero temperature allowed a better maintenance of quality and an extended shelf-life, from five to 12 days, than a counterpart batch stored in flake ice. Sensory analysis clearly revealed better scores in the external and flesh odor, skin aspect, consistency, gills and eyes in hake stored in slurry ice. Accordingly, significantly lower counts of total aerobes and proteolytic bacteria, and lower increases in the pH value and in both TMA-N and TVB-N concentrations. The good results obtained in the microbiological, sensory and chemical analyses reported in this work clearly confirm the practical advantages of using slurry ice for the refrigeration and moderately long chilled storage of a lean fish species of relevant commercial value such as European hake.

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Figure captions

Fig 1. Comparative evolution of total aerobes in hake muscle during storage in either flake ice (□) or slurry ice (◇).

Fig 2. Comparative evolution of proteolytic bacteria in hake muscle during storage in either flake ice (□) or slurry ice (◇).

Fig. 3. Comparative evolution of the pH value in hake muscle during storage in either flake ice (□) or slurry ice (◇).

Fig. 4. Comparative evolution of total volatile base-nitrogen (TVB-N) content in hake muscle during storage in either flake ice (□) or slurry ice (◇).

Fig. 5. Comparative evolution of trimethylamine-nitrogen (TMA-N) content in hake muscle during storage in either flake ice (□) or slurry ice (◇).

Fig. 1

Fig. 2

Fig. 3

Fig. 4

Fig. 5

1 Table 1 **Modificar interlineado**

2 Scale employed for evaluating the freshness of European hake along storage

Attribute	Highest quality (E)	Good quality (A)	Fair quality (B)	Unacceptable (C)
Skin	Very intense pigmentation; transparent mucus	Milky mucus; insignificant pigmentation losses	Slightly greyish mucus; pigmentation without shine	Widely opaque mucus; important pigmentation losses
External odor	Sharply seaweedy and shellfish	Weakly seaweedy and shellfish	Incipiently sour and putrid	Sour and putrid
Gills	Brightly red; without odor; lamina perfectly separated	Rose colored; without odor; lamina adhered in groups	Slightly pale; incipient fishy odor; lamina adhered in groups	Grey-yellowish color; intense ammonia odor; lamina totally adhered
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
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 odor	Sharply seaweedy and shellfish	Weakly seaweedy and shellfish	Incipiently sour and putrid	Sour and putrid

1 Table 2 ¿Apaisado mejor?

2 Comparative sensory acceptability of European hake batches^a

		Slurry ice batch								Flake ice batch					
		(days of storage)								(days of storage)					
	0	2	5	8	12	15	19	22	2	5	8	12	15	19	
Skin aspect	E	E	A	A	B	B	B	C	E	A	A	B	C	C	
External odor	E	A	A	A	A	B	C	C	E	A	B	C	C	C	
Gills	E	A	A	A	B	B	B	B	A	B	C	C	C	C	
Eyes	E	A	A	A	B	C	C	C	A	B	B	C	C	C	
Consistency	E	A	A	A	A	A	C	C	A	A	B	B	B	C	
Flesh odor	E	A	A	A	B	B	B	B	A	A	B	B	C	C	

3 ^a Freshness categories are as expressed in Table 1.

Table 3 Cambiar formato

1 Average counts^a of anaerobes and coliforms in hake muscle stored in either slurry ice or flake ice

	0	Slurry ice batch (days of storage)							Flake ice batch (days of storage)					
		2	5	8	12	15	19	22	2	5	8	12	15	19
Anaerobes	2.28±1.32	1.27±1.24	0.33±0.67	1.77±1.77	2.02±1.15	2.51±0.61	1.36±0.11	2.38±0.52	1.96±0.12	0.96±0.96	1.59±0.29	2.33±1.07	3.08±0.24	2.92±0.62
Coliforms	0	0	0	0	0.59±1.18	0	0	0.68±1.36	0	0	0	0	1.79±0.32	0.86±0.86

2 ^aResults are expressed as log CFU/g. Values represent mean±range.

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