1 Sensory, microbial and chemical effects of a slurry ice system on horse mackerel (Trachurus
2 trachurus)
3

4 **Desired section:** Sensory and nutritive qualities of food

5

6 Title:

7 **Extended Shelf Life of Horse Mackerel (*Trachurus trachurus*) Chilled**

8 **in Slurry Ice as Determined by Sensory and Microbiological Analysis.**

9

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25 **Short title:** Extended shelf life of horse mackerel in slurry ice

26 Horse mackerel shelf-life in slurry ice

1   **ABSTRACT**

2

3   Slurry ice, a biphasic system consisting of small spherical ice crystals surrounded by

4   seawater, was evaluated in parallel to flake ice for the storage of horse mackerel

5   (*Trachurus trachurus*). Storage in slurry ice implied a significant enhancement of the

6   shelf life (5d for flake ice to 15d for slurry ice), better control of pH value, and lower

7   counts of total aerobes, proteolytic and lipolytic bacteria, these reaching average

8   differences between batches of 2, 1.43 and 1.98 log units, respectively, after 8 d of

9   storage. Storage in slurry ice also implied significantly slower formation of total volatile

10   base-nitrogen and trimethylamine after 8 d of storage. *Staphylococcus xylosus* and

11   *Proteus penneri* were identified as the leading proteolytic and lipolytic organisms in

12   horse mackerel muscle. Storage of horse mackerel in slurry ice enhances the shelf life of

13   this medium-fat fish species through a better maintenance of sensory and

14   microbiological quality.

15

16

17   **Key Words:** Horse mackerel; Slurry ice; Shelf life; Sensory quality; Microbiological

18   activity; Proteolysis; Lipolysis.

1 **INTRODUCTION**

2

3 Aquatic food products deteriorate rapidly due to the joint action of microbiological,
4 enzyme and chemical spoilage mechanisms (Pigott and Tucker 1987; Hsieh and
5 Kinsella 1989). During chilled storage damage to the fish is slowed down but not
6 prevented, this leading to losses in sensory quality and nutritional value (Whittle and
7 others 1990). While traditional chilling has involved the use of flake ice (Nunes and
8 others 1992) or refrigerated seawater (Kraus 1992), more recently, slurry ice –also
9 known as fluid ice, slush ice, liquid ice or flow ice– has been introduced as a promising
10 technique for the preservation of fish products at subzero temperature.
11 Slurry ice can be defined as a biphasic system consisting of small spherical ice
12 crystals suspended in iced water at a temperature slightly above the initial freezing point
13 of fish (0ºC to -2ºC). Among the main advantages of slurry ice, two should be
14 highlighted: its faster chilling rate –due to its higher heat-exchange capacity–, and the
15 limited physical damage that causes to fish food products –due to the spherical
16 geometry of its microscopic ice crystals–. Other advantages of slurry ice derive from its
17 complete coverage of the fish surface, which affords a better protection of the fish
18 surface with respect to oxidation and dehydration events. Slurry ice can also be pumped,
19 this guaranteeing a more hygienic handling of the fish products, and may be combined
20 with other agents, such as ozone, to achieve an antiseptic surface effect, or melanosis
21 inhibitors, to prevent browning reactions in shellfish (Huidobro and others 2002).
22 Chapman (1990) reported a better maintenance of quality of finfish stored on-board
23 in slurry ice as compared with other chilling methods, a result similar to that found for
24 the on-board storage of albacore tuna by other authors (Price and others 1991). Harada
25 (1991) also underlined the advantages of slurry ice as a pre-cooling method for fish. The
26 scientific literature recently accounts for the use of slurry ice systems for the storage of
27 Australian prawns (Chinivasagam and others 1998), and shrimp (Huidobro and others
28 2002). Other authors have also reported that slurry ice represents a good slaughter
29 method to sacrifice and store farmed seabream (Huidobro and others 2001).
30 Horse mackerel is a medium-fat species abundant in Northeast Atlantic (FAO 1998;
31 Aubourg and Ugliano 2002) and has a potential role in the prevention of heart disease
32 due of its high content in PUFAs (n-3) not synthetized by humans. Horse mackerel has
33 not been extensively utilized as raw material for the fish industry in the past, but is
34 deserving an increasing attention from fish technologists, being currently considered as
35 an infra-utilized fish species with a high commercial potential as an effective functional
36 food (García and others 1996; Tabara and others 1998). The minimal seasonal variation
37 of the horse mackerel lipids was reported by Bandarra and others (2001), who also
38 underlined the nutritional interest of this fish species as an important yr-round source of
39 lipids of dietary importance. Previous research carried out on chilling storage work
40 reports biochemical analyses (amine formation and lipid damage) (Aubourg, 2001) and
41 physico-chemical parameters (Monteagudo-Torres and others 2002), although
42 microbiological parameters were not considered.
43 In this work we have applied an advanced slurry ice system to the storage of horse
44 mackerel (*Trachurus trachurus*) during 22 d, and compared with a control batch stored
45 in parallel in conventional flake ice. With a view to investigating the shelf life of horse
46 mackerel, here the effects of storage of horse mackerel in slurry ice on sensory and
47 microbiological quality were investigated during 22 d. In addition, the isolation and
48 identification of major bacteria involved in the proteolytic and lipolytic breakdown of
49 horse mackerel muscle was also undertaken.

1 **MATERIALS AND METHODS**

2

3 **Slurry ice and flake ice systems**

4   A slurry ice prototype (FLO-ICE, Kinarca S.A.U., Vigo, Spain) was used in the

5 present work. The composition of the slurry ice binary mixture was 40% ice and 60%

6 water, prepared from filtered seawater (salinity: 3.3%). The temperature of the slurry ice

7 mixture was -1.5ºC. Flake ice was prepared with an Icematic F100 Compact device

8 (CASTELMAC SPA, Castelfranco, Italy).

9

10 **Fish material, processing and sampling**

11   Specimens of horse mackerel (*Trachurus trachurus*) were caught during the day at a

12 local fishing bank close to Northwestern Spain and kept on ice until they arrived at our

13 laboratory. The fish specimens were neither headed nor gutted. The length of the

14 specimens was in the range of 16-21 cm; the weight was in the range of 230-270 g. The

15 fish specimens were placed in either slurry or flake ice at a fish:ice proportion of 1:1,

16 and stored for up to 22 d in a refrigerated room at 2ºC. When required, the flake ice and

17 the slurry ice mixture were renewed.

18   For each chilling treatment, three different batches were used and studied separately

19 along the whole experimental period. Samples were taken from each batch on days 0, 2,

20 5, 8, 12, 15, 19 and 22. Once the intact specimens had been subjected to sensory

21 analyses, the white muscle was separated and used for microbiological and chemical

22 analyses; all analyses were performed in triplicate.

23

24

25 **Sensory analyses**

1   Sensory analyses were conducted by a taste panel consisting of five experienced

2   judges, according to the guidelines presented in Table 1 (DOCE 1989). Four categories

3   were ranked: highest quality (E), good quality (A), fair quality (B) and unacceptable

4   (C). Sensory assessment of the fish included the following parameters: skin, external

5   odor, gills, eyes, consistency and flesh odour.

6

7   **Microbiological analyses**

8   Samples of 25 g of fish muscle were dissected aseptically from chilled horse

9   mackerel specimens, mixed with 225 ml of 0.1% peptone water, and homogenised in a

10   stomacher (Seward Medical, London, UK) as previously described (Ben-Gigirey and

11   others 1998, 1999). For assays at abusive temperatures, whole fish fillets were placed

12   inside sterile bags and kept at 30ºC for 3 d before the fish extracts were prepared. In all

13   cases, serial dilutions from the microbial extracts were prepared in 0.1% peptone water.

14   Total aerobes were investigated in plate count agar (PCA, Oxoid Ltd., London, UK)

15   after incubation at 31ºC for 72 h. Anaerobes were investigated in the same way, except

16   that an anaerobic atmosphere kit (Oxoid) was placed together with the plates inside the

17   anaerobiosis jar. Lactose-fermenting *Enterobacteriaceae* (coliforms) were investigated

18   in Violet Red Bile Agar (VRBA medium, Merck, Darmstadt, Germany) after incubation

19   at 30ºC±1ºC for 24±2 h, as recommended by the manufacturer (Merck Microbiology

20   Manual, 2002).

21   Microorganisms exhibiting a proteolytic phenotype were investigated in casein-agar

22   medium (30ºC/48 h) (Phaff and others 1994), as previously described (Ben-Gigirey and

23   others 2000). Bacterial colonies exhibiting a lipolytic phenotype were detected in

24   tributyrine-agar medium, as described elsewhere (Ben-Gigirey and others 2000).

1   **Table 1 – Scale employed for evaluating the freshness of horse mackerel.**

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 odour	Sharply seaweedy and shellfish smell	Weakly seaweedy and shellfish smell	Incipiently putrid and rancid	Putrid and rancid
Gills	Brightly red; without odour; lamina perfectly separated	Rose coloured; without odour; lamina adhered in groups	Slightly pale; incipient fishy odour; lamina adhered in groups	Grey-yellowish colour; intense ammonia odour; 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 odour	Sharply seaweedy and shellfish smell	Weakly seaweedy and shellfish smell	Incipiently putrid and rancid	Putrid and rancid

1   Routine microbiological tests included the investigation of colony morphology, cell

2   morphology, motility, Gram stain, and production of cytochrome oxidase and catalase,

3   as described elsewhere (Rodríguez and others 2003a). The identification of major

4   proteolytic and lipolytic bacterial strains was accomplished using miniaturized

5   biochemical tests: API 20 E and API 20 NE for Gram-negative microorganisms, and

6   API 50CH and API STAPH for Gram-positive microorganisms, all of them from

7   BioMèrieux (Marcy L'Etoile, France). The results of the identification tests were

8   interpreted using the APILAB PLUS software (BioMèrieux). The enzymic profiles of

9   the proteolytic and lipolytic bacterial isolates were further characterized using the API

10   ZYM system (BioMèrieux).

11

Chemical analyses

13   The evolution of pH values along the storage time was carried out using a 6-mm

14   diameter insertion electrode (Crison, Barcelona, Spain). Total volatile base-nitrogen

15   (TVB-N) values were measured according to Aubourg and others (1997). On it, fish

16   muscle (10 g) is extracted with perchloric acid (6%) and made up to 50 ml, the TVB-N

17   content being determined –after steam-distillation of the acid extracts rendered alkaline

18   to pH 13 with NaOH (20%)– by titration of the distillate with 10 mM hydrochloric acid.

19   The results are expressed as mg TVB-N/100 g muscle. Trimethylamine-nitrogen (TMA-

20   N) values were obtained by the Tozawa and others (1971) method, which involves the

21   preparation of a 5% trichloracetic acid extract of fish muscle (10 g/25 ml) and reaction

22   with picric acid. Data are expressed as mg TMA-N/100 g muscle.

23

1 **Statistical analyses**

2   Bacterial counts were transformed into log CFU/g before subjecting to statistical

3 analyses. Data from the different chemical measurements were subjected to one-way

4 analysis of variance; comparison of means was performed using a least-significant-

5 difference (LSD) method (Statsoft 1994). The SPSS 11.5 software for Windows (SPSS

6 Inc., Chicago, IL) was also used to explore the statistical significance of the results

7 obtained, this including multivariate contrasts and multiple comparisons by the Scheffé

8 and Tukey tests. A confidence interval at the 95 % level ($p<0.05$) was considered in all

9 cases.

1 **RESULTS AND DISCUSSION**

2

3 **Sensory analyses**

4   According to the results of the sensory analyses (Table 2), horse mackerel stored in

5 slurry ice maintained good quality (E and A categories) up to day 8, while the

6 counterpart batch stored in flake ice only maintained such quality up to day 2. As

7 storage time progressed, sensory quality decreased and by day 8 (flake ice batch) and

8 day 19 (slurry ice batch), the specimens were no longer acceptable. The external

9 features that limited the acceptability of the flake ice batch were: the external odour, the

10 flesh odour and the gills. The shelf life of horse mackerel stored in flake ice determined

11 in this study agreed with previous works carried out with this fish species (Simeonidou

12 and others 1997; Aubourg 2001) and with other small fish species such as sardine

13 (Nunes and others 1992) and mackerel (Bennour and others 1991). It should be

14 remarked the considerable increase of shelf life obtained as a consequence of the use of

15 slurry ice employment, the horse mackerel specimens keeping an acceptable quality at

16 least until day 15. A similar enhancement of shelf life as a consequence of the use of

17 slurry ice has been recently reported for lean fish species such as turbot (Rodríguez and

18 others 2003b) or hake (Rodríguez and others 2003c).

19

20 **Quantitative microbiological analyses**

21   Figures 1, 2 and 3 display the most relevant results concerning microbial growth in

22 horse mackerel stored in either slurry ice or flake ice. Statistically significant ($p < 0.05$)

23 differences were observed between both batches for aerobes, proteolytic and lipolytic

24 bacteria. In the case of total aerobes, average difference in the counts between batches

1   **Table 2 – Comparative sensory evaluation of horse mackerel batches.[a]**

|	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**	**22**
Skin aspect	E	E	A	A	B	C	C	E	A	B	C	C	C	C
External odour	E	A	A	B	B	C	C	E	A	C	C	C	C	C
Gills	E	E	A	B	B	C	C	E	B	C	C	C	C	C
Eyes	E	A	A	B	B	C	C	E	A	B	B	C	C	C
Consistency	E	E	A	A	B	B	B	E	A	B	B	B	C	C
Flesh odour	E	A	A	B	B	C	C	E	A	C	C	C	C	C

2   [a] Freshness categories are as expressed in Table 1. Initial quality at day 0 deserved an "E" score (highest quality) for all parameters.
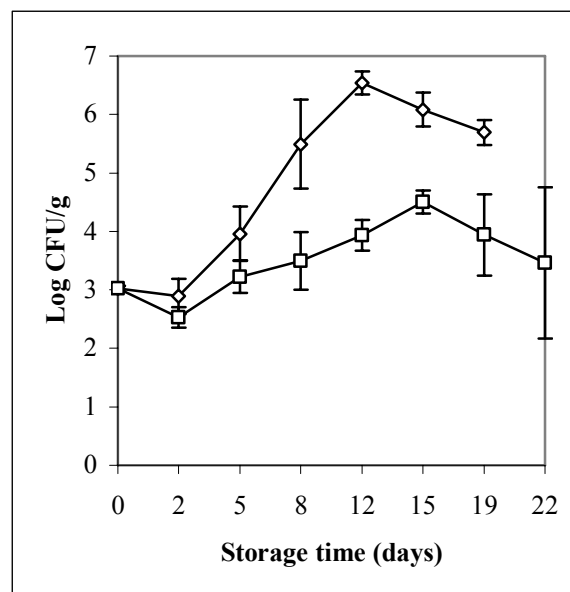
1   **Figure 1 – Comparative evolution of total aerobes in horse mackerel muscle during**

2   **storage in either slurry ice (□) or flake ice (◊).**

1   was 2.0 log units on day 8, and this difference even increased to 2.61 log units after 12 d

2   of storage (Figure 1). At both sampling times the flake ice batch had exhibited

3   unacceptable quality according to sensory evaluation, but the slurry ice batch still

4   maintained acceptable quality (Table 2). The total aerobic counts reached levels close to

5   10^6 CFU/g in the flake ice batch after 8 d of storage, although these numbers were

6   below those considered by other authors to provoke the spoilage of fish stored

7   aerobically (Gram and Huss 1996). Storage of horse mackerel in slurry significantly

8   reduced bacterial growth, this may contributing to the enhanced shelf life determined by

9   sensory evaluation. These results agree with recent works reporting significantly lower

10   bacterial counts in shrimp (Huidobro and others 2002), turbot (Rodríguez and others

11   2003b), and hake (Rodríguez and others 2003c) stored in slurry ice, as compared with

12   counterpart batches stored in conventional flake ice. A recent work has reported aerobic

13   bacteria counts as high as 10^7 CFU/g in the skin of horse mackerel stored for 7 d at 4ºC

14   (Kuda and others 2002). In this sense, the surface wash caused by the liquid phase of

15   the slurry ice together with the subzero temperature achieved with this advanced storage

16   system, may be argued as the two main reasons of the limited bacterial growth found in

17   the muscle of horse mackerel in the slurry ice batch, as compared with the flake ice

18   batch.

19   Microbial metabolites such as peptides or amino acids derive from protein

20   hydrolysis and may also contribute significantly to undesirable sensory changes in

21   seafood products. It is well known that such modifications in odour, texture and

22   appearance are directly related to spoilage in aquatic food products (Shewan, 1977;

23   Makarios-Laham and Lee 1993; Rodríguez and others 2003a). Asakawa and others

24   (1998) characterized a protease from a *Bacillus* sp. strain isolated from horse mackerel,

25   and which was thought to play a major role in post-mortem decomposition of skin
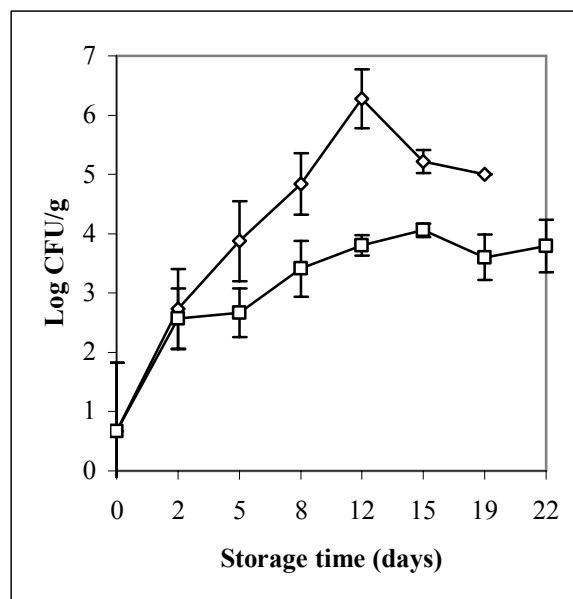
1   **Figure 2 – Comparative evolution of proteolytic bacteria in horse mackerel muscle**

2   **during storage in either slurry ice (□) or flake ice (◊).**

1   tissue, this leading to flesh spoilage. Microbial proteolysis of muscle has also been

2   reported to cause sensory spoilage in horse mackerel homogenates stored at 10ºC

3   (Kobatake and others 1992). In our work, the evolution of the counts of microorganisms

4   potentially involved in the proteolytic breakdown of horse mackerel was investigated in

5   both batches. As displayed in Figure 2, statistically significant ($p<0.05$) lower counts of

6   proteolytic bacteria were determined in the muscle of horse mackerel kept in slurry ice,

7   as compared with flake ice. The average difference in the counts between batches was

8   1.43 log units on day 8, and this difference even increased to 2.48 log units after 12 d of

9   storage (Figure 2). The numbers of proteolytic bacteria in the muscle of horse mackerel

10   stored in flake ice reached levels above 10^6 CFU/g by day 12, while the slurry ice batch

11   only reached counts of 10^4 CFU/g or lower, even on day 22, this clearly indicating a

12   significant decrease in the growth of this bacterial group in horse mackerel muscle

13   stored in slurry ice. The results presented here confirm other previous studies indicating

14   a significantly slower growth of proteolytic bacteria in turbot (Rodríguez and others

15   2003b), and hake (Rodríguez and others 2003c) stored in slurry ice, as compared with

16   flake ice.

17   The medium-fat nature of horse mackerel makes this fish species especially

18   sensitive to mechanisms involved in lipid damage (Aubourg and Ugliano 2002). Among

19   these mechanisms, the production of extracellular proteases by certain microorganisms

20   may play a role in the lipolytic breakdown of fish species such as albacore tuna (Ben-

21   Gigirey and others, 2000). Accordingly, the evolution of the numbers of

22   microorganisms exhibiting lipolytic activity was investigated in both batches, the results

23   being presented in Figure 3. Statistically significant ($p<0.05$) lower numbers of lipolytic

24   bacteria were determined in the slurry ice batch than in the flake ice batch. On day 8,

25   the average difference in the numbers between both batches was found to be 1.98, this

26   difference increasing up to 4.40 log units after 12 d of storage (Figure 3). The numbers
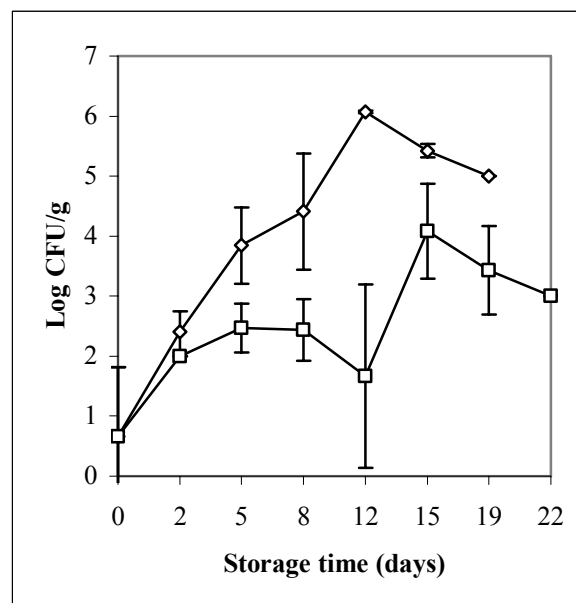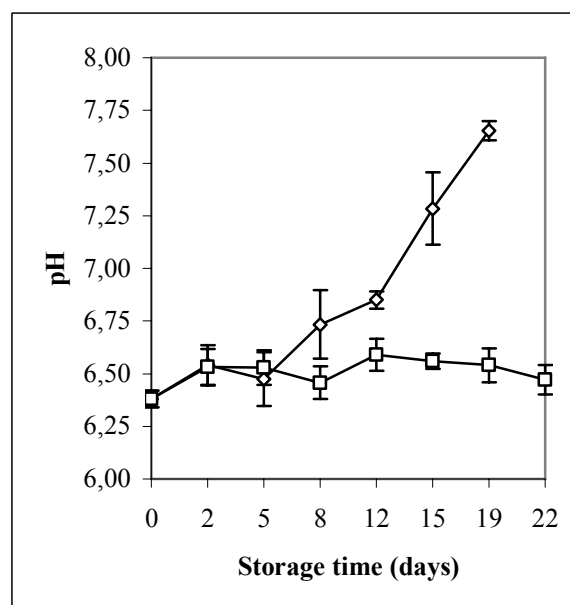
1 **Figure 3 – Comparative evolution of lipolytic bacteria in horse mackerel muscle**

2 **during storage in either slurry ice (□) or flake ice (◊).**

1 of lipolytic bacteria in the muscle of horse mackerel stored in flake ice reached levels

2 above 10^6 CFU/g by day 12, while the slurry ice batch only reached counts of 10^4

3 CFU/g after 15 d of storage. According to these results, the growth of bacteria

4 potentially involved in the lipolytic breakdown of horse mackerel was slowed down as a

5 consequence of storage in slurry ice. This is, to our knowledge, the first results

6 describing the effects of slurry ice on microbial lipolytic activity in a medium-fat fish

7 species such as horse mackerel.

8 The average counts of anaerobes in muscle of horse mackerel stored in slurry ice

9 were 2.5 log CFU/g, no statistically significant at the $p < 0.05$ level being determined

10 between both batches. The evolution of anaerobes in each batch throughout storage

11 neither evidenced significant differences with respect to the initial counts at day 0 (2.16

12 log CFU/g). These results confirm the notably good initial quality of the fish specimens

13 and the limited growth of anaerobes during chilled storage, regardless of the ice system

14 employed. With respect to the development of coliforms, similar results were obtained.

15 Thus, the average numbers of coliforms throughout storage were very low (< 1 log

16 CFU/g), no statistically significant ($p < 0.05$) difference being observed between both

17 batches, and neither with respect to the initial counts at day 0. Similar low numbers of

18 anaerobes and coliforms had been obtained by Figueroa and others (1990) in jack

19 mackerel.

20 It should also be remarked that the counts of total aerobes, proteolytic and lipolytic

21 bacteria, correlated well with the differences observed in the sensory evaluation, a result

22 that also agreed with previous reports for shrimp (Huidobro and others 2002), turbot

23 (Rodríguez and others 2003b), and hake (Rodríguez and others 2003c) kept in slurry

24 ice. The slowing down effect of storage in slurry ice on the growth of proteolytic and

25 lipolytic bacteria, according to the results presented here, would imply a lower presence

26 of microbial proteases and lipases in the muscle of horse mackerel, this limiting the

1 **Figure 4 − Comparative evolution of pH value in horse mackerel muscle during**

2 **storage in either slurry ice (□) or flake ice (◊).**

1   negative effects of such resistant enzymes on the lipid compounds of this fish species,

2   since proteases and lipases may retain activity for long periods even at low temperatures

3   (Alford and Pierce 1961).

4

5   **Chemical analyses**

6   Statistically significant ($p < 0.05$) differences were observed for the pH value in horse

7   mackerel stored in slurry ice and flake ice (Figure 4). Thus, while only slight increases

8   in pH (from the initial 6.38 to a peak of 6.59) were observed in the slurry ice batch,

9   significant pH increases (up to a pH value of 7.65) were determined in the flake ice

10   batch along storage. The remarkable increase of pH in the flake ice batch might indicate

11   a more intense growth of alkalinizing bacteria in such batch, this leading to a higher

12   accumulation of ammonia compounds, with the subsequent negative effects on sensory

13   quality, especially external and flesh odour..

14   The different evolution of the pH value observed in each batch correlated well with

15   the evolution of TVB-N and TMA-N (Figures 5 and 6, respectively). Thus, the

16   formation of both TVB-N and TMA-N in horse mackerel muscle was slowed down in

17   the slurry ice batch, especially after 8 d and 5 d of storage, respectively. Thus, The

18   TVB-N content (Figure 5) of horse mackerel stored in slurry ice was very low, reaching

19   levels of 31 mg/100 g after 22 days of storage. By contrast, the TVB-N content

20   exhibited a dramatic increase after 8 d of storage in the muscle of horse mackerel stored

21   in flake ice, reaching concentrations as high as 84 mg/100 g after 19 days of storage.

22   Statistical analysis confirmed that the storage of horse mackerel in slurry ice implied a

23   significantly ($p < 0.05$) lower formation of TVB-N as compared with flake ice. Other

24   authors have also reported TVB-N contents higher than 50 mg/100 g in horse mackerel

25   stored for 9 d at 4ºC (Kuda and others 2002), this also being far above those determined

26   in this study in horse mackerel stored in slurry ice.

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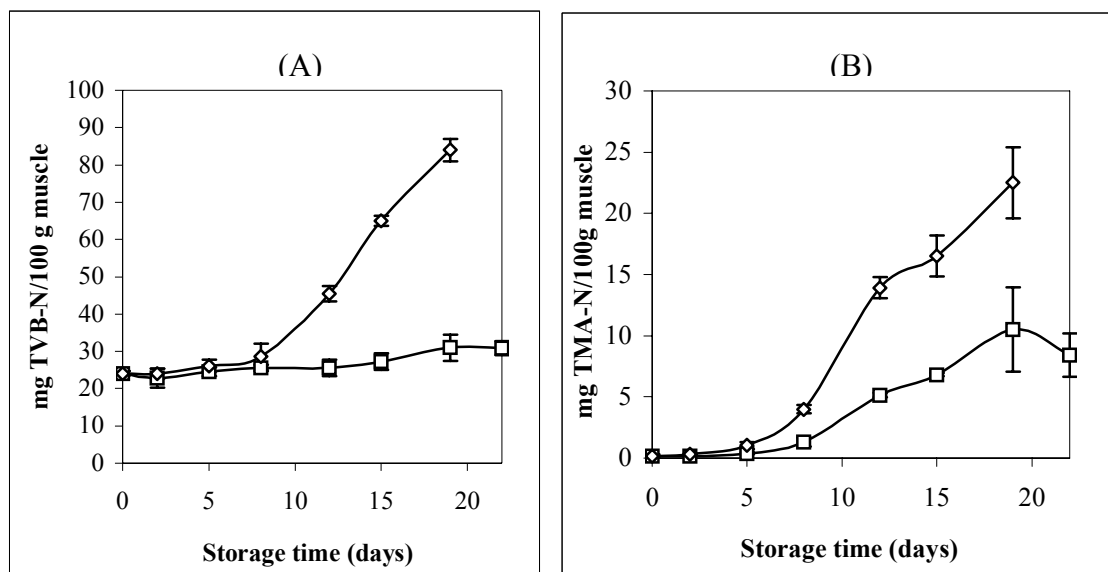
Figure 5 – Determination of (A) total volatile base-nitrogen (TVB-N) and (B) trimethylamine-nitrogen (TMA-N) contents in horse mackerel muscle during storage in either slurry ice (□) or flake ice (◊).

1   Likewise, the TMA-N content in muscle also increased very slowly in the period

2   between 0-22 days of storage in the slurry ice batch. After day 5, a sharp increase of the

3   TMA-N content was determined only in the muscle of horse mackerel stored in flake

4   ice. Finally, average TMA-N values as different as 10.5 and 22.5 mg/100 g were

5   determined in the slurry ice batch and flake ice batch, respectively, after 19 days of

6   storage (Figure 5B). As expected from the results obtained in the present study, storage

7   in slurry ice significantly ($p<0.05$) slowed down the formation of TMA-N, especially

8   after 5 d of storage, in comparison with storage in flake ice. Since TMA-N has been

9   reported to the best chemical parameter to determine quality loss on horse mackerel

10   (Aubourg 2001), the benefits of slurry ice for extending the shelf life of horse mackerel

11   should be remarked.

12

13   **Identification of bacteria potentially involved in the proteolytic and lipolytic**

14   **breakdown of horse mackerel muscle**

15   There is little previous information available about the identification of spoilage

16   microorganisms from horse mackerel. Silva and others (1998) studied effects of

17   inoculation of five spoilage species into horse mackerel stored under and ozone

18   atmosphere, but several of the microbial species considered were typical spoilage

19   bacteria, not specific microorganisms isolated from horse mackerel. Thus, a qualitative

20   analysis of the predominant proteolytic and lipolytic bacterial strains isolated from

21   horse mackerel muscle stored in liquid ice during 22 d and then subjected to abusive

22   temperatures (30ºC) for 3 d was carried out in this work.

23   From the 26 initial microbial isolates exhibiting proteolytic or lipolytic activity in

24   plate bioassays, 13 isolates showing different phenotypes in the preliminary

25   microbiological study and relative abundance of the total proteolytic or lipolytic

26   colonies in each fish specimen were selected for further study (Table 3). Except for

1 **Table 3 – Identification of microorganisms involved in the proteolytic and lipolytic**

2 **breakdown of horse mackerel muscle.**

Strain	Storage conditions	Proteolytic (P)/ Lipolytic (L) activity[a]	Bacterial species
P1	Slurry ice + 30ºC[b]	P (++)/L (++)	*Staphylococcus xylosus*
P7	Slurry ice + 30ºC[b]	P (++)/L (–)	*Staphylococcus xylosus*
P8	Slurry ice + 30ºC[b]	P (+)/L (++)	*Proteus penneri*
P9	Slurry ice + 30ºC[b]	P (++)/L (+)	*Proteus penneri*
P13	Slurry ice + 30ºC[b]	P (++)/L (++)	*Staphylococcus xylosus*
L1	Slurry ice + 30ºC[b]	P (+++++)/L (+)	*Proteus penneri*
L2	Slurry ice + 30ºC[b]	P (+++++)/L (++)	*Staphylococcus xylosus*
L5	Slurry ice + 30ºC[b]	P (+++)/L (+++)	*Proteus penneri*
L7	Slurry ice + 30ºC[b]	P (+++)/L (+++)	*Proteus penneri*
L8	Slurry ice + 30ºC[b]	P (+++)/L (+++)	*Proteus penneri*
L9	Slurry ice + 30ºC[b]	P (+++)/L (+++)	*Staphylococcus xylosus*
L12	Slurry ice + 30ºC[b]	P (+++++)/L (++)	*Staphylococcus xylosus*
L13	Slurry ice + 30ºC[b]	P (++++)/L (++)	*Proteus vulgaris*

3 [a] Proteolytic/lipolytic activity: + = very weak; ++ = weak; +++ = moderate; ++++ =

4 strong; +++++ = very strong.

5 [b] Isolates were obtained from muscle stored in slurry ice for 22 d and then subjected to

6 abusive temperature conditions for 3 d.

1   strain P7, that only exhibited a proteolytic phenotype, all the remaining 12 isolates

2   exhibited both proteolytic and lipolytic activities. Six isolates were identified as

3   *Staphylococcus xylosus*, while other six belonged to the species *Proteus penneri* –an

4   indol-negative variant of *Proteus vulgaris*– the other isolate being identified as *Proteus*

5   *vulgaris* (Table 3). While *Proteus* spp. have been previously isolated from a number of

6   aquatic food products, *S. xylosus* strains have deserved attention of fish technologists

7   because of its ability to biosynthetize and secrete extracelular histidine decarboxylase,

8   this leading to the formation of histamine in seafood products such as semi-preserved

9   anchovies (Rodríguez-Jerez and others 1994).

10   The phenotypic differences among the proteolytic and lipolytic isolates belonging to

11   the same species were also investigated by the API ZYM system. The results obtained

12   for *S. xylosus* isolates indicated that all the six strains could be classified in three

13   groups: (i) a trypsine-producing, weak proteolytic/weak lipolytic group (isolates P1 and

14   P13), (ii) a trypsine non-producing, strong proteolytic/weak lipolytic group (isolates L2,

15   L9 and L12), and (iii) a trypsine non-producing, moderate proteolytic/non-lipolytic

16   strain (isolate P7). Accordingly, it was concluded that at least three different *S. xylosus*

17   strains, these were P1, L2 and P7, belonged to the microflora of horse mackerel, the

18   other *S. xylosus* strains being multiple isolates of any of such three strains. Interestingly,

19   all *S. xylosus* strains isolated from horse mackerel biosynthesized and secreted alkaline

20   phosphatase, acid phosphatase, leucine aryl amidase, naphtol-phosphohydrolase and α-

21   glycosidase.

22   With respect to the *P. penneri* strains, two of them –P8 and P9– produced cisteine

23   aryl amidase, showed a weak production of extracellular lipases and proteases and

24   almost equivalent phenotypic profiles. Isolates L5, L7 and L8 of *P. penneri* did not

25   produce cisteine aryl amidase and exhibited moderate proteolytic/moderate lipolytic

26   activity. Finally, isolate L1 did not produce cisteine aryl amidase and exhibited very

1 strong proteolytic/weak lipolytic activity. Thus, at least three different *P. penneri* strains

2 –P8, L5 and L1– were isolated from horse mackerel under abusive temperature

3 conditions. Interestingly, all *P. penneri* strains isolated from horse mackerel also

4 biosynthethized and secreted alkaline phosphatase, acid phosphatase, leucine aryl

5 amidase, naphtol-phosphohydrolase and α-glycosidase.

6 Thus, the results obtained in this work suggest that *P. penneri* and *S. xylosus* are

7 involved in the proteolytic and lipolytic breakdown of horse mackerel muscle. In

8 addition to these two species, a moderately lipolytic/strong proteolytic strain of *Proteus*

9 *vulgaris* was also isolated. The fact that these three species produce glycosidic enzymes

10 can enhance their proteolytic and lipolytic activity. Thus, such enzymes, together with

11 their bacterial proteases and lipases, can degrade cell membranes and expose proteins

12 and lipids to the respective action of proteases and lipases (Marin and Marshall 1983;

13 Marin and others 1984). The fact that slurry ice significantly slowed down the growth of

14 proteolytic and lipolytic bacteria in the muscle of horse mackerel, as described above,

15 underlines the benefits that such storage system may exert on the maintenance of quality

16 and shelf life of this fish species.

1 **CONCLUSIONS**

2

3 In summary, from the results of the sensory analyses presented here, storage of horse

4 mackerel in slurry ice allows a better maintenance of quality and enhances the shelf life

5 of this fish species more than 10 d: from 5 d (flake ice batch) to 15 d (slurry ice batch).

6 Storage in slurry ice was accompanied by significantly lower counts of total aerobes,

7 proteolytic and lipolytic bacteria, better control of pH and a slowed down formation of

8 both TMA-N and TVB-N. The good results obtained in the microbiological, sensory,

9 and chemical analyses strongly suggest the use of slurry ice to improve the chilled

10 commercialization of horse mackerel.

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