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.

Running Title: Quality and safety in Grand Sole fish

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 (Whittle, Hardy, & Hobbs, 1990; Olafsdóttir et al., 1997). Unlike other muscle food, fish are usually harvested in remote locations, this making the time between the catch and the landing of the fish material much longer than the time between landing and selling in the shop (Ward, 1994). As a result of this, the threat of having fish condemned, withdrawn from sale, or sold at low prices at harbour, rather than the capacity of the fish room, limits the length of the voyage (Kelman, 1982).

Bacterial growth during storage increases with handling and due to the direct contact with decks, equipment and boxes (Huss, Dalsgaard, Hansen, Ladefoged, Pedersen, & Zitan, 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 to spoilage. With the recent trend of fresh seafood consumption in the European countries, public health concerns have become an issue requiring careful attention, not only to ensure product quality but also its safety. The potential health risks associated with fresh seafood, together with the high demand far from local fishing ports, makes the need for long-term preservation techniques to be addressed continually (Ashie, Smith, & Simpson, 1996).

In this sense, and 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 in an ice-water suspension at subzero temperatures (Yamada, Fukusako, & Kawanami, 2002). Its use has proven to slow down microbial growth, this leading 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 monk, *Lophius piscatorius*; thornback ray, *Raja clavata*) from the Grand Sole North Atlantic fishing bank. This distant bank, exploited by several European countries, is so far that the time elapsed between the catch and arrival at destiny varies from 10 to 15 days. In order to optimise the fish quality and 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

A SI prototype (FLO-ICE, Kinarca S.A.U., Vigo, Spain) was installed in the ship *Patricia-Marta*, based on Vigo fishing harbour (Northwestern Spain). The composition of the SI binary mixture was 40% ice and 60% water, prepared on-board

with filtered seawater (salinity: 3.3%). The temperature of the SI mixture was –1.5ºC. FI was prepared on-board using freshwater with an Icematic F100 Compact device (Castelmac SPA, Castelfranco, Italy); the temperature of the FI was –0.5ºC.

2.2. Fish material, processing and sampling

European hake (*Merluccius merluccius*; length 30-35 cm, weight 170-210 g), angler (*Lophius piscatorius*; length 37-45 cm, weight 190-230 g) and thornback ray (*Raja clavata*; length 40-55 cm, weight 330-370 g) were captured in the Grand Sole North Atlantic fishing bank throughout a single trip. Hake and angler were gutted immediately after catching, while ray was not. None of the fish species was headed. For each fish species, individuals were distributed on-board into SI and FI treatments. For it, 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 ºC. Each fish species was captured at four different times of the trip, and at each time, individuals were separated into three groups that were analysed separately in order to achieve the statistical study (n=3).

Once the fish specimens were unloaded at Vigo fishing harbour, they were transported to the laboratory and kept in an isothermal room (0-1 ºC) 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 from catching. 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 sensory 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 inoculation in plate count agar (PCA, Oxoid) after incubation at 30ºC for 72 h. Psychrotrophes were also investigated in PCA (Oxoid) but incubation was carried out at 7-8ºC for 10 days. *Enterobacteriaceae* were investigated in Crystal Violet Neutral Red Bile Glucose Agar (VRBD Agar) (Merck, Darmstadt, Germany) after incubation at 37ºC for 24 h. Microorganisms exhibiting a proteolytic phenotype were investigated in casein-agar medium after incubation at 30ºC 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 (AOAC, 1990). Results were expressed as g NaCl kg^{-1} flesh 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 kg^{-1} flesh 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 muscle (10 g/ 25 ml). The results were expressed as mg TMA-N kg^{-1} flesh muscle.

2.6. Statistical analyses

Bacterial counts were transformed into log CFU g^{-1} flesh muscle before undergoing statistical analysis. Data corresponding to both chilling methods were subjected to one-way analysis of variance to asses significant ($p<0.05$) differences between treatments. The SPSS 11.5 for Windows software (SPSS Inc., Chicago, IL, USA) was also used to explore the statistical significance of the results obtained, this including multivariate contrasts and 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 kg^{-1} flesh muscle) for hake, angler, and ray, respectively. Moisture values were in the ranges 783-804, 821-833 and 774-796 (g kg^{-1} flesh muscle) for hake, angler and ray, respectively. For each fish species, statistical analysis evidenced that the differences found in both constituents 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 evidenced 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 the 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 much lower than those reported for fish muscle refrigerated and stored in seawater (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 provided in all cases values lower than 10 CFU g^{-1} flesh muscle, no significant ($p>0.05$) 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 discarded.

With respect to the counts of total aerobes (Figure 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 provide significant ($p>0.05$) differences as a result of storage time, while hake and ray specimens led to an increasing ($p<0.05$) tendency with storage time. In all cases, aerobe counts reached levels slightly above 10^4 CFU g^{-1}, 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 psychrotrophic bacteria (Figure 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 of angler fish, where no significant differences ($p>0.05$) were observed between both icing conditions. In all cases, an increasing ($p<0.05$) tendency with time was observed, so that counts slightly below 10^6 and around 10^5 CFU g^{-1} were obtained for hake on one hand, and for angler and ray on the other, respectively.

For the three fish species studied, the investigation of the proteolytic bacteria (Figure 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 the time of storage were observed.

3.3. Chemical analyses of microbial activity

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

In the case of 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 SI system; however, this index did not provide an increasing tendency with storage time in any case. Such 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 in the case of fish stored under FI conditions. As a result, a higher ($p<0.05$) total volatile amine formation was concluded in ray fish kept under FI conditions.

Marine elasmobranches such as ray produce and retain within their bodies large amounts of urea (Read, 1968; Finne, 1992). The high levels of TVB-N content found for ray in the present study can be explained as a consequence of microbial decomposition of urea into ammonia (Vyncke, 1978; Finne, 1992). In this sense, evaluation of the TVB-N values corresponding to the different fish species tested in the present experiment (Table 3) led to higher levels ($p<0.05$) 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 than in the other two fish species under study. For

hake and angler, a significant ($p<0.05$) formation of trimethylamine (TMA) was observed along storage time in both chilling systems, being this formation higher ($p<0.05$) in the case of 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 such two species stored under FI conditions, according to the end of the lag phase. Results on TMA formation in hake are in agreement with previous research (Ruiz-Capillas & Moral, 2001; Baixas-Nogueras et al., 2002), 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 later quantifies a wide range of basic volatile compounds (ammonia, methylamine, dimethylamine, trimethylamine, etc.) produced by different damage pathways, while TMA-N assessment accounts only for the trimethylamine oxide breakdown.

On contrast, when ray fish specimens are considered (Table 4), significant differences in TMA-N values are not observed ($p>0.05$) 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 ray values were found markedly higher than those obtained in the two other species under study; this result agrees to the higher TMAO amounts found in cartilaginous fishes than in other kinds of fish (Read, 1968; Finne, 1992).

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 would

be the elimination of waste ammonia that otherwise would reach toxic concentrations, being accomplished this elimination 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 an increasing ammonia formation. According to its above-mentioned function, TMAO content would be gradually lost with the increasing damage of the ray specimens. Consequently, a TMA formation decrease would occur as ray damage increases. The experimental data provided in this study strongly support this theory. Thus, the deviation of TMAO to the ammonia elimination would limit the amount of TMAO available as a substrate of the bacterial activity, this 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 showed the worst scores were: consistency and gill cavity (hake), eyes (angler) and external ammonia odour and consistency (ray).

By 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 throughout the storage time agreed to the evolution of chemical (TMA-N value) and most microbiological (aerobe, psychrotrophe, and proteolytic counts) parameters. For ray fish, the sensory quality loss is in accordance with the TVB-N value and the microbiological parameters. The time of sensory quality rejectance in angler and ray stored under FI conditions agreed 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) gave longer shelf-life times (20-25 days). In the present work, the shelf-life was not this long, being explained by the relative smaller size of the hake specimens employed in our study.

4. CONCLUDING REMARKS

According to microbial, chemical, and sensory analyses, the use of SI instead of FI as chilling system caused a partial inhibition of microbial activity, the former system implying a better maintenance of quality and an extended shelf life of the fish specimens investigated. In agreement to the on-board availability of SI formation, 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 while unloading, this allowing increased commercial values during its 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 of angler specimens during their chilled storage.

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FIGURE CAPTIONS

Figure 1: Total aerobe assessment* in fish (hake, angler, and ray) muscle during storage in 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).

Figure 2: Psychrotrophic bacteria assessment* in fish (hake, angler, and ray) muscle during storage in different chilling conditions **

* Mean values of three independent determinations (n = 3) are presented; bars denote standard deviations.

** Chilling conditions as expressed in Figure 1.

Figure 3: Proteolytic bacteria assessment* in fish (hake, angler, and ray) muscle during storage in different chilling conditions **

* Mean values of three independent determinations (n = 3) are presented; bars denote standard deviations.

** Chilling conditions as expressed in Figure 1.

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

TABLE 2

NaCl content (g kg^{-1} flesh 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 indicated in brackets.

** Chilling conditions: FI (flake ice) and SI (slurry ice).

*** Chilled storage times expressed in brackets correspond to ray fish experiment.

TABLE 3

Changes in total volatile base-nitrogen (TVB-N) content* (mg TVB-N kg^{-1} flesh 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 indicated in brackets.

** Chilling conditions as expressed in Table 2.

*** Chilled storage times expressed in brackets correspond to ray fish experiment.

TABLE 4

Changes in trimethylamine-nitrogen (TMA-N) content* (mg TMA-N kg^{-1} flesh 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 indicated in brackets.

** Chilling conditions as expressed in Table 2.

*** Chilled storage times expressed in brackets correspond to ray fish experiment.

TABLE 5

Sensory assessment* of fish species stored under different chilling conditions**

Chilled storage time (days)***	Hake		Anglerfish		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

* Freshness categories as expressed in Table 1.

** Chilling conditions as expressed in Table 2.

*** Chilled storage times expressed in brackets correspond to ray fish experiment.

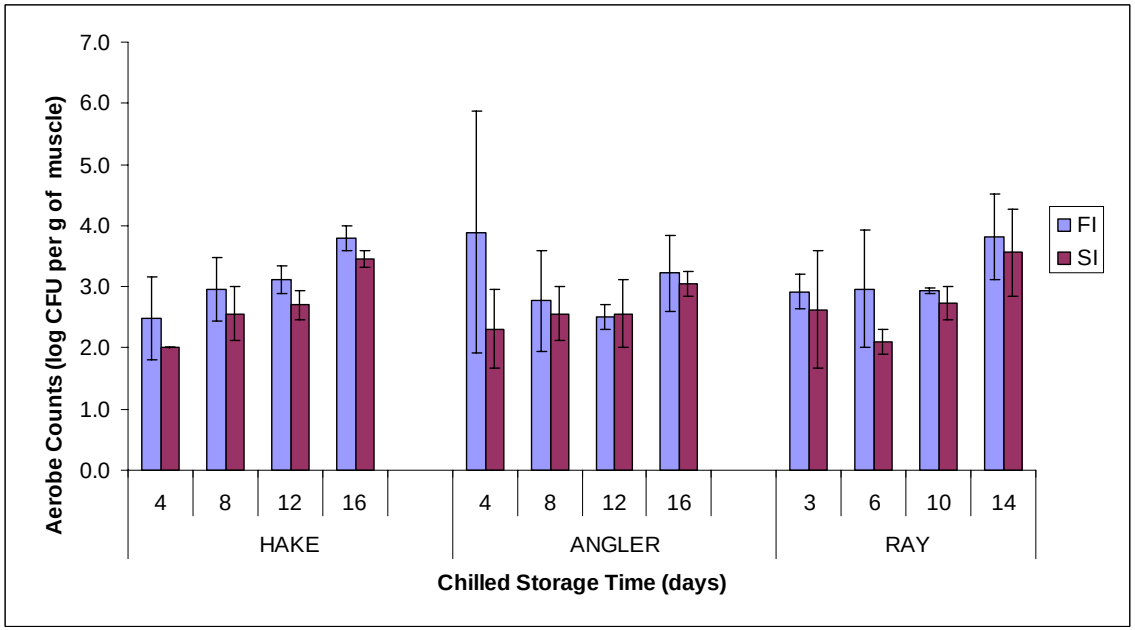
7.0
6.0
5.0
4.0
3.0
2.0
1.0
0.0
Aerobe Counts (log CFU per g of muscle)
FI
SI
4
8
12
16
4
8
12
16
3
6
10
14
HAKE
ANGLER
RAY
Chilled Storage Time (days)

Figura 1

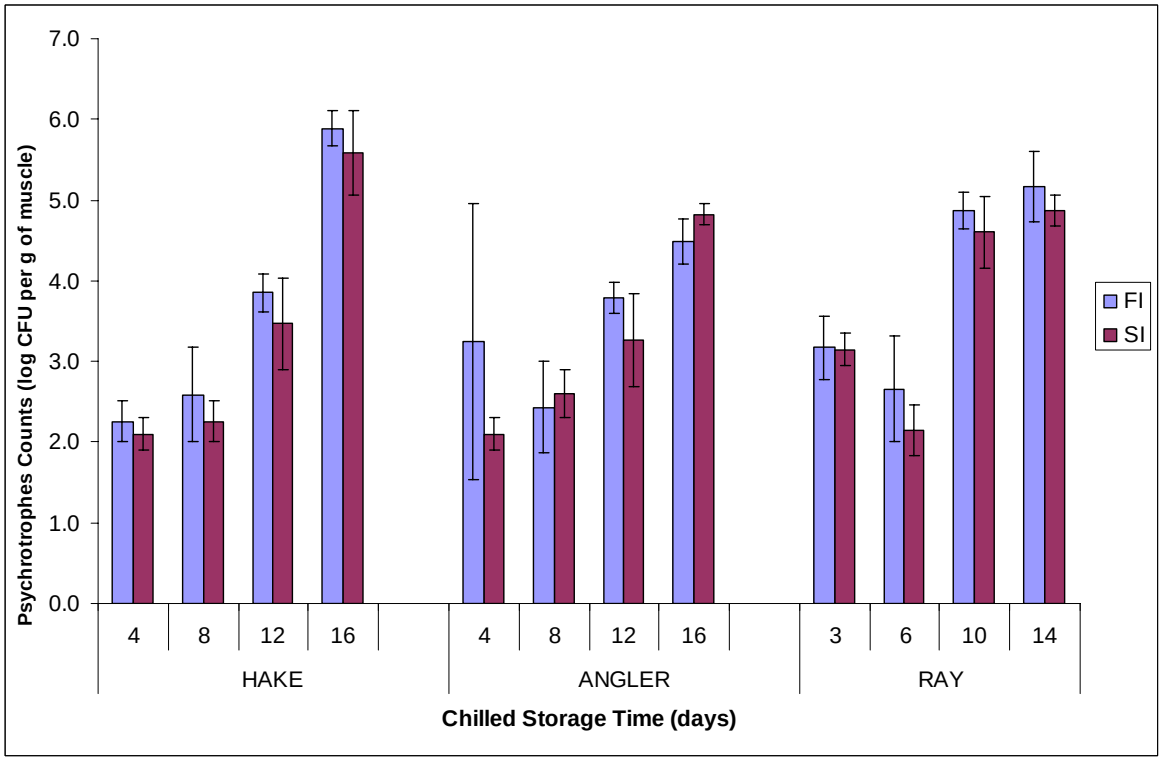
7.0
6.0
5.0
4.0
3.0
2.0
1.0
0.0
Psychrotrophes Counts (log CFU per g of muscle)
4
8
12
16
4
8
12
16
3
6
10
14
HAKE
ANGLER
RAY
Chilled Storage Time (days)
FI
SI

Figura 2

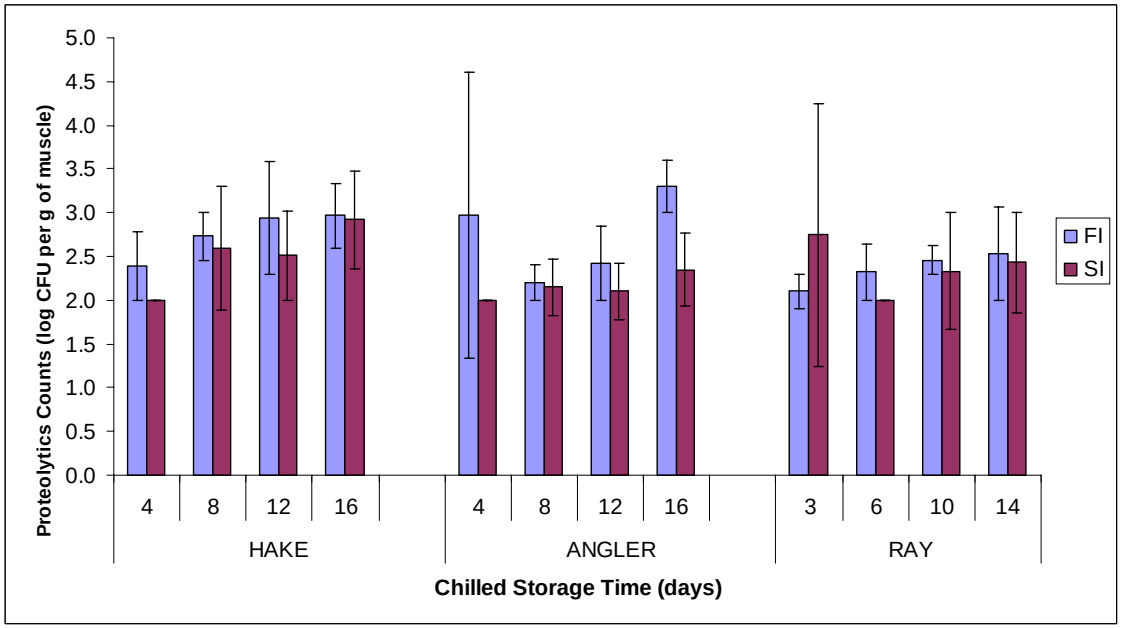
Proteolytics Counts (log CFU per g of muscle)
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
4
8
12
16
4
8
12
16
3
6
10
14
HAKE
ANGLER
RAY
Chilled Storage Time (days)
FI
SI

Figura 3