Inhibition of quality loss in chilled megrim 
(*Lepidorhombus whiffiagonis*) by employing citric 
and lactic acid icing

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SUMMARY

This study focuses on the quality retention of megrim (*Lepidorhombus whiffiagonis*) during chilled storage. Aqueous solutions of two different concentrations of citric (CA) and lactic (LA) acids were employed as icing media (0.125% CA-0.050% LA and 0.175% CA-0.050% LA, respectively; w/v). The effects of each solution on microbial activity, lipid damage and sensory acceptance were monitored for up to 13 d of storage. Lower (p<0.05) bacterial growth was detected according to microbiological (aerobe and psychrotroph counts) and chemical (trimethylamine-N and pH) assessments, which led to an enhancement of sensory appreciation. Whereas control fish were determined as unacceptable at day 13, the acid-iced fish were still acceptable at that time. Concerning lipid damage, an inhibitory effect (p<0.05) on fluorescent compound formation was observed in the acid-iced fish. Present results allow to conclude that the use of a CA-LA icing system can provide a profitable strategy to obtain higher quality chilled fish.

**Keywords**: *Lepidorhombus whiffiagonis*, chilling, citric and lactic, microbial activity, sensory acceptance.

**Running Head**: Citric-lactic icing and chilled megrim
INTRODUCTION

Marine foods are considered to be an important source of beneficial components for human health and nutrition (Simopoulos, 1997). Most of the beneficial constituents, however, deteriorate rapidly upon capture or harvest and the degree to which the degradation process continues depends directly on storage conditions. Flake-ice chilling has been the most commonly used method to retard fish damage. However, deterioration of sensory quality and nutritional value has been detected in chilled fish as a result of microbial and biochemical degradation mechanisms (Whittle et al., 1990; Kyrana & Lougovois, 2002). According to the actual need for high-quality fresh products, flake ice has been combined with other preservative strategies, such as previous chemical (Özogul et al., 2011) or physical (Ashie et al., 1996) treatment, the employment of protective packaging (Sivertsvik et al. 2002) and the presence of preservative compounds (ozone; Pastoriza et al., 2008) and plant extracts (Quitral et al., 2009; Özyurt et al., 2012) in the icing medium.

In this scenery, natural low molecular weight organic acids and their sodium salts represent a relevant choice because of their easy availability, low commercial cost and wide range of permitted concentrations for their use. Such compounds have directly been added to fish products, or have been included in aqueous solutions where fish samples have been dipped for a concrete time before subsequent storage or processing. Among such organic acids, citric acid (CA) is widely known for its role as a chelator and acidulant in biological systems and its use has led to a profitable effect on fish fillet (Badii & Howell 2002; Pourashouri et al., 2009) and whole fish (Aubourg et al., 2004) quality. Furthermore, lactic acid (LA) has been reported to be effective in preserving and extending the shelf-life of fish fillets (Metin et al., 2001; Kilinc et al., 2009), coated fish (Gogus et al., 2006) and fish slices (Sallam, 2007). Recently, both acids have been
successfully included **with other components** in a commercial formula, **whose aqueous solution** has been applied as a previous dipping **treatment and** as icing medium during blue whiting (*Micromesistius poutassou*) chilled storage (Sanjuás-Rey *et al*., 2011).

Flatfish (e.g., flounder, sole, turbot, plaice and halibut) represent a very important food group. Another flatfish, megrim (*Lepidorhombus whiffiagonis*), is abundant in the Northeast Atlantic waters. This species, which is considered one of the most fished species in the Grand Sole North Atlantic Fishing bank, has been exploited by a wide number of European countries, including the United Kingdom, France, Ireland and Spain (FAO, 2007). Its capture at such a distant fishing bank usually means that the time elapsed between the catch and arrival to destination can vary from 10 to 15 d. This example highlights the need to optimise refrigeration parameters to provide consumers with fish of the highest possible quality.

Previous research on megrim has demonstrated that the quality of the fish diminishes during storage and the effects of different on-board storage conditions have been tested (Jehano *et al*., 1996; Aubourg *et al*., 2006). In addition, the development of different damage pathways has been analysed, including volatile amine formation during refrigeration (1-3°C) (Civera *et al*., 1995) and increased microbial development (Sanjuás-Rey *et al*., 2012) and lipid oxidation (García-Soto *et al*., 2011) during chilled storage. The present research focuses on the quality retention of megrim during chilled storage. To enhance megrim quality, aqueous solutions of CA and LA were employed as an icing medium and the effects on microbial activity inhibition, lipid oxidation stability and sensory acceptance were monitored in megrim muscle for up to 13 d of chilled storage.
MATERIALS AND METHODS

Icing systems

Two aqueous solutions with CA and LA concentrations (w/v) of 0.125% and 0.050% (condition C-125) and 0.175% and 0.050% (condition C-175), respectively, were prepared. Both solutions were packed in polythene bags and kept frozen at –20°C until use. Traditional ice was prepared using water (condition C-0; control) that was packed and kept frozen in the same way as the organic acid mixtures. The different ices were ground to obtain common flakes before their addition to the individual fishes. The organic acids used in the present research are generally recognised as safe (GRAS) for use in foods according to European and American standards (Madrid et al., 1994; Giese, 1996).

A preliminary study was undertaken to elucidate the concentrations of CA and LA to be used for the ice preparation in the present research. For it, solutions combining both acids in concentrations of 0.005-0.250 % (w/v) were applied to megrim and compared to fish kept under traditional flake ice by means of sensory analysis. Thus, different sensory descriptors (namely, skin, eyes and consistency) were analysed by a sensory panel as described in the sensory analysis sub-section (guidelines concerning fresh and refrigerated fish; Council Regulation, 1990). As a result, the concentrations used for the C-125 and C-175 conditions in the present research were chosen as being the highest concentrations that did not lead to significant changes in appearance.

Fish material, processing and sampling

Fresh megrim (*Lepidorhombus whiffiagonis*) (117 individuals) were caught near the Galician Atlantic coast (northwestern Spain) and transported on ice to the laboratory
10 h after they were caught. The length and weight of the fish specimens ranged from
20 to 23 cm and 95 to 120 g, respectively.

Upon arrival in the laboratory, 9 individual fishes were separated and analysed as starting raw fish (day 0). These fish were divided into three different groups (three individuals per group) and were analysed independently to achieve the statistical analysis (n=3). The remaining fish were divided into three batches (36 individuals in each batch), placed in boxes and directly surrounded by one of the three types of ices previously mentioned (conditions C-0, C-125 and C-175). A 1:1 fish-to-ice ratio was employed and all of the batches were placed in a refrigerated room (4ºC). Boxes that allowed draining were used and the ice was renewed when required. Fish samples from all of the batches were analysed on days 2, 6, 9 and 13. At each sampling time, nine total individuals were analysed and divided into three groups from each batch (three individuals in each group) that were studied independently (n=3). Sensory analysis was carried out on the whole fish, whereas microbiological and chemical analyses were carried out on the white muscle.

**Sensory analysis**

Sensory analysis was conducted by a sensory panel that consisted of five experienced judges who adhered to traditional guidelines concerning fresh and refrigerated fish, which was adapted to megrim (Council Regulation, 1990). The panellists had participated in the sensory analysis of various fish and seafood products for the previous ten years. Before carrying out the present experiment, the judges received special training on chilled megrim. This training focused on the evaluation of refrigerated specimens that exhibited different qualities. Special attention was paid to
the evolution of the different sensory parameters from the initial state to the state in which the sensory attributes were no longer acceptable.

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 descriptors: skin and mucus development, eyes, external odour, gill appearance and odour, consistency, flesh odour (raw and cooked) and flesh taste (cooked). Fish samples were individually scored by the panellists.

**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 (Merck, Darmstadt, Germany) and homogenised in sterilised stomacher bags (AES, Combourg, France) as previously described (Ben-Gigirey et al., 1998; Ben-Gigirey et al., 1999). In all of the cases, serial dilutions from the microbial extracts were prepared in 0.1% peptone water.

Total aerobes were investigated via surface inoculation on plate count agar (PCA, Oxoid Ltd., London, UK) after incubation at 30°C for 48 h. The anaerobe counts were also determined in PCA at 30°C; however, an anaerobic atmosphere kit (Oxoid) was placed along with the plates inside the anaerobiosis jar. Psychrotrophs were also investigated in PCA, but the incubation was carried out at 7-8°C for 7 d. Enterobacteriaceae were investigated via pour plating using Violet Red Bile Agar (VRBA) (Merck, Darmstadt, Germany) after incubation at 37°C for 24 h. Microorganisms that exhibited a proteolytic phenotype were investigated in casein agar medium after incubation at 30°C for 48 h, as previously described by Ben-Gigirey et al. (2000). In all of the cases, bacterial counts were transformed into log CFU g⁻¹ muscle before undergoing statistical analysis. All of the analyses were conducted in triplicate.
Chemical analyses

The evolution of pH values in megrim muscle over the storage time was determined using a 6-mm diameter insertion electrode (Crison, Barcelona, Spain).

Trimethylamine-nitrogen (TMA-N) values were determined using the picrate method, as previously described by Tozawa et al. (1971). This method 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\(^{-1}\) muscle.

Lipids were extracted from the fish white muscle by the Bligh and Dyer (1959) method, which employs a single-phase solubilisation of the lipids using a chloroform-methanol (1:1) mixture. The results were calculated as g lipid kg\(^{-1}\) muscle.

Free fatty acid (FFA) content was determined using the lipid extract of the fish muscle by the Lowry and Tinsley (1976) method, which is based on complex formation with cupric acetate-pyridine followed by spectrophotometric (715 nm) assessment. The results are expressed as g FFA kg\(^{-1}\) lipids.

The peroxide value (PV) was determined spectrophotometrically (Beckman Coulter, DU 640, London, UK) using the lipid extract via previous peroxide reduction with ferric thiocyanate according to the Chapman and McKay (1949) method. The results are expressed as meq active oxygen kg\(^{-1}\) lipids.

The thiobarbituric acid index (TBA-i) was determined according to Vyncke (1970). This method is based on the reaction between a trichloroacetic acid extract of the fish muscle and thiobarbituric acid. The content of thiobarbituric acid reactive substances (TBARS) was spectrophotometrically measured at 532 nm and calculated from a standard curve using 1,1,3,3-tetraethoxy-propane (TEP). The results are expressed as mg malondialdehyde kg\(^{-1}\) muscle.
The formation of fluorescent compounds (Fluorimeter LS 45; Perkin Elmer España, Tres Cantos, Madrid, Spain) was determined by measurements at 393/463 nm and 327/415 nm as described by Aubourg (1999). The relative fluorescence (RF) was calculated as follows: \( RF = \frac{F}{F_{st}} \), where \( F \) is the fluorescence measured at each excitation/emission maximum and \( F_{st} \) is the fluorescence intensity of a quinine sulphate solution (1 µg ml\(^{-1}\) in 0.05 M H\(_2\)SO\(_4\)) at the corresponding wavelength. The fluorescence ratio (FR) was calculated as the ratio between the two RF values: \( FR = \frac{RF_{393/463 \text{ nm}}}{RF_{327/415 \text{ nm}}} \). The FR value was determined using the aqueous phase that resulted from the lipid extraction of the fish muscle (Bligh & Dyer, 1959).

**Statistical analysis**

The data that were obtained from the different microbial and chemical analyses were subjected to the ANOVA method to explore differences resulting from the effects of both the icing conditions and the chilling time. This analysis was carried out using the PASW Statistics 18 software for Windows (SPSS Inc., Chicago, IL, USA). The comparison of means was performed using the least-squares difference (LSD) method. Differences between batches and among icing times were considered significant for a confidence interval at the 95% level (\( p<0.05 \)) in all cases.

**RESULTS AND DISCUSSION**

**Microbiological analyses**

The evolution of aerobic mesophiles in megrim muscle is shown in Table 1. Statistically significant (\( p<0.05 \)) differences between the control batch (C-0) and the C-125 and C-175 batches were determined at days 9 and 13, respectively. Remarkably, the two batches with CA and LA in the icing systems did not reach aerobic counts of 7 log
CFU g\(^{-1}\) after 9 d of chilled storage, whereas the control batch widely surpassed this limit. The greatest difference between the batches was determined for the C-0 and C-125 batches on day 9 (i.e., rising to 1.82 log CFU g\(^{-1}\)). The results demonstrate that the presence of CA and LA in the icing medium protect the fish from aerobic mesophiles.

The investigation of psychrotrophs did not reveal any significant (p>0.05) differences among the batches during the first 6 d of chilled storage (Table 1). In contrast, significant (p<0.05) differences among the batches were detected after longer storage times. Indeed, a remarkable inhibition of psychrotrophs was observed on days 9 and 13. The C-175 batch did not reach 6 log CFU g\(^{-1}\) counts even after 13 d of storage, whereas the number of psychrotrophs in the control group rose above 7 log CFU g\(^{-1}\) on day 9. The greatest differences between the C-125 and C-175 batches and the control batch were 1.45 and 2.32 log units, respectively, which indicated that the organic acids provided marked protection against microbes in the megrim muscle.

With respect to the anaerobes, these did not exhibit significant (p>0.05) differences among batches at any storage time (Fig. 1). At advanced storage times, however, lower mean values of the anaerobes growth were observed in the C-125 and C-175 batches, although the differences were not significant. None of the batches exhibited anaerobe counts above 6 log CFU g\(^{-1}\), even after 13 d of chilled storage. The evolution of Enterobacteriaceae revealed low counts for this microbial group at all storage times (Fig. 2). Only the C-125 batch on day 9 provided numbers higher than 3 log CFU g\(^{-1}\). Indeed, the other batches and storage times were always below that limit, even after 13 d of chilled storage. The present study also investigated the development of proteolytic bacteria. The role of proteolytic bacteria in the breakdown of fish muscle has been previously highlighted (Rodríguez et al., 2003; Campos et al., 2005). The obtained results are presented in Fig. 3 and are quite similar to those obtained for
anaerobes. Indeed, no significant (p>0.05) difference among batches was observed at any storage time. Similar to the observations for the anaerobes, however, lower mean values of proteolytic bacteria formation were observed in the C-125 and C-175 batches at advanced storage times, although such differences were not significant.

In global terms, it can be concluded that incorporating CA and LA into flake ice for the chilled storage of megrim allowed for a better control of microbial growth in megrim muscle compared with conventional flake ice. Indeed, this effect was significant (p<0.05) in the case of aerobic mesophiles and psychrotrophs (Table 2), where the organic acids provided a better maintenance of the microbial quality of megrim throughout storage. The present study complements previous studies concerning related technologies that have focused on the preservation of megrim and other marine species under advanced refrigeration systems, such as slurry ice. Slurry Ice technology has been shown to result in slower microbial growth in fish muscle due to the washing effect of the salt solution on the fish surface during ice melting (Losada et al., 2004; Campos et al., 2005). In the present work, the melting of the flake ice crystals containing the natural CA and LA solutions might exert a similar washing effect that may reduce the surface microbial load and its diffusion towards the muscle.

The present study is supported by previous studies that have been carried out in our laboratory to evaluate a novel icing system that consists of a commercial formula that includes an aqueous solution of CA, LA and ascorbic acid (AA). The use of this acidic solution has been shown to help control aerobic mesophiles in megrim as well as in hake (Merluccius merluccius) and angler (Lophius piscatorius) (Sanjuás-Rey et al., 2012). In addition, lower counts of aerobe and psychrotroph bacteria were obtained in blue whiting (Micromesistius poutassou) muscle (Sanjuás-Rey et al., 2011) by applying the same commercial formula in a two-step processing strategy (previous dipping and
ice storage). Similar to our results, other authors have reported that the inclusion of natural preservative compounds in the icing system can effectively inhibit microbial activity. For example, studies have shown the effectiveness of a rosemary extract during chilled storage of sardines (*Sardinella aurita*) (Özyurt *et al*., 2012) and a wild-thyme hydrosol extract in the chilled storage of Transcaucasian barb (*Capoeta capoeta capoeta*) (Oral *et al*., 2008).

**Chemical analyses**

Scarce differences were observed in the pH values throughout the chilled storage for each of the different batches considered (Table 2). Indeed, the pH values were similar for all cases and all values remained under 7.0, which is considered acceptable for certain species, such as hake (Aubourg *et al*., 2006). The mean pH values were higher in the control fish than in the treated batches (C-125 and C-175) and the differences were significant (p<0.05) at days 2 and 13. No differences (p>0.05) were found between the two acid concentrations used for the icing condition tests.

In agreement with the present pH results, the employment of a commercial formula including CA, LA and AA in the icing system did not cause any changes in the pH value throughout the chilled storage of hake muscle (Sanjuás-Rey *et al*., 2012). However, other studies have reported a pH decrease as a result of using natural preservative compounds in ice during the chilled storage of other marine species. These studies include the use of oregano and rosemary extracts during the chilled storage of Chilean jack mackerel (*Trachurus murphyi*) (Quitral *et al*., 2009), a rosemary extract applied to sardines (Özyurt *et al*., 2012) and a wild-thyme hydrosol extract employed in the chilled storage of Transcaucasian barb (Oral *et al*., 2008).
An increasing trimethylamine (TMA) formation (p<0.05) was observed in samples from each batch as a result of chilled storage (Table 2). This increase was markedly significant on day 13 and supports previous research, which has shown a high TMA formation in megrim muscle during chilled storage (Civera et al., 1995; Aubourg et al., 2006; Sanjuás-Rey et al., 2012). A lower TMA formation (p<0.05) was observed after applying the highest acid concentration in the icing system, which suggests that the icing condition had an inhibitory effect. Previous research has also shown the inhibitory effect of including preservative components in the icing system on TMA content. For example, the presence of CA, LA and AA in a commercial formula that was used in an icing system resulted in an inhibitory effect on the TMA content in hake and angler muscle during their chilled storage (Sanjuás-Rey et al., 2012). In addition, the application of slurry ice (Aubourg et al., 2006) and ozonated ice (Pastoriza et al., 2008) led to a lower TMA content in megrim muscle during its on-board chilled storage.

A progressive formation (p<0.05) of FFA was observed with chilling time in samples from each batch (Table 2) and the values that were attained agree with previous research that showed a high FFA formation in megrim muscle during chilled storage (Aubourg et al., 2006; García-Soto et al., 2011). Some significant differences (p<0.05) were observed as a result of the icing system employed; however, a definite trend concerning the icing effect could not be concluded because a higher formation was observed in the C-125 batch during the 6-9-day period, whereas batch-175 fish revealed the highest FFA content at day 13.

FFA formation in fish during chilled storage has been reported to be a result of endogenous enzyme activity and microbial activity (Whittle et al., 1990; Ashie et al., 1996). Before the end of the microbial lag phase (up to 6-9 days, depending on several factors), FFA formation should mostly result from endogenous enzyme (namely lipases...
and phospholipases) activity. After the microbial lag phase, microbial activity should become more significant as a result of bacterial catabolic processes. In addition to these FFA formation pathways, a FFA breakdown process is brought about by bacterial lipoxygenases (Kyrana & Lougovois, 2002), which is more significant in longer chilled storage times. The fact that a definite trend concerning the acid-icing effect on FFA content was not found in the present research could be explained by different, opposite FFA formation/breakdown mechanisms.

Similar to our results, an inhibitory effect on lipid hydrolysis was not observed by Özyurt et al. (2012), who employed a rosemary extract during the chilled storage of sardines. Contrary to our results, an inhibitory effect on fish FFA formation could be observed by applying a rosemary and oregano extract to chilled Chilean jack mackerel (Quitral et al., 2009). In addition, a lower FFA formation was obtained in blue whiting muscle after a two-step process that consisted of dipping and icing fish with a commercial formula that contained CA, LA and AA (Sanjuás-Rey et al., 2011).

Peroxide formation (primary oxidation) increased (p<0.05) in samples from all of the batches throughout the chilled storage (Table 3). The values cannot be considered especially high because they remained below a score of 8.50 in all of the cases. Significant differences resulting from the icing systems could not be concluded (p>0.05); however, higher mean values were observed in the C-175 batch between days 6 and 13.

TBARS formation (secondary oxidation) was also relatively low because values were under a score of 0.80 in all cases (Table 3). Samples from all of the batches showed a progressive TBA-i increase (p<0.05) with chilling time. No significant differences (p>0.05) were obtained as a result of the icing system applied; however,
higher mean values were observed in fish corresponding to the C-125 batch in the 6-13-day period.

The assessment of fluorescent compound formation showed a general increase with time (p<0.05) for all of the fish batches studied (Table 3). The acid presence displayed an inhibitory effect on day 13 for both of the acid concentrations that were tested. Additionally, fish corresponding to the control batch had higher mean values than acid-iced fish throughout the whole experiment.

Lipid oxidation is not considered a major damage pathway during chilled storage of lean fish (lipid content range in the present experiment: 4.8-6.5 g kg\(^{-1}\) muscle) and the present study confirmed that there was low primary and secondary lipid oxidation, as previously reported (Whittle \textit{et al.}, 1990; Sanjuás \textit{et al.}, 2011). The electrophilic character of such lipid oxidation compounds has led them to interact with food constituents that possess nucleophilic functions (Howell, 1995; Aubourg, 1999) to form fluorescent compounds (tertiary lipid oxidation compounds). Interestingly, the control fish had the highest FR value, which suggests a significant effect of the acid in the ice.

Previous research has shown that including natural preservative compounds in the icing system creates an inhibitory effect on fish lipid oxidation. For example, lipid oxidation was inhibited in Chilean jack mackerel as a result of including an oregano and rosemary extract in the icing system (Quitral \textit{et al.}, 2009) and sardines by including a rosemary extract (Özyurt \textit{et al.}, 2012). Directly related to the present research, the employment of a commercial formula including CA, LA and AA led to a lower oxidation development during the chilled storage of different fish species, including hake, megrim, and angler (García-Soto \textit{et al.}, 2011) and blue whiting (Sanjuás-Rey \textit{et al.}, 2011).
**Sensory analyses**

Evaluation of the different sensory descriptors was carried out in all types of samples and the results are expressed in Table 4. A progressive score decrease with chilling time was found in all of the batches of fish. An inhibitory effect of the acid presence in the ice on sensory quality loss could be concluded because the control fish were found to be unacceptable at day 13, whereas the acid-treated fish (C-125 and C-175 batches) were still acceptable at that time. Limiting descriptors were external odour, flesh odour (raw and cooked) and flesh taste. There were no differences in the gills or consistency between the different icing systems.

Very similar scores were given to fish corresponding to both of the acid-icing conditions; however, the C-125 fish had better scores than the C-175 fish (i.e., skin (day 9) and eyes (day 6) appearance). This difference may be explained as a slight effect of the higher acid concentration on the external appearance of the fish; however, fish samples corresponding to the C-175 condition were evaluated as acceptable by the panel in both cases.

An increased shelf life has also previously been observed as a result of including natural preservative compounds in the icing system. Indeed, the shelf life of chilled megrim, hake and angler (Sanjuás et al., 2012) and blue whiting (Sanjuás-Rey et al., 2011) has been extended by applying a commercial formula including CA, LA and AA in the ice. The same result was obtained with the use of a rosemary extract during chilled storage of sardines (Özyurt et al., 2013), oregano and rosemary extracts during the chilled storage of Chilean jack mackerel (Quitral et al., 2009) and a wild thyme hydrosol extract in chilled Transcaucasian barb (Oral et al., 2008).
CONCLUSIONS

Previous research has reported the positive effects of including preservative compounds in the icing system on quality retention in chilled lean fish. The previous studies evaluated commercial formulae and plant extracts. In the present study, two organic natural acids (CA and LA) that are widely known for their easy availability and low commercial cost have been considered. The employment of an aqueous solution of both acids as the icing medium inhibited deterioration mechanisms and subsequently enhanced the quality of megrim during chilled storage. Microbiological (namely aerobe and psychrotroph counts) and chemical (namely TMA-N and pH) assessments demonstrated that the use of both acids in the icing system inhibited bacterial growth and enhanced sensory appreciation. As a result, acid-iced fish were still acceptable at day 13, while control fish showed a shelf life time of 9 d. Concerning lipid deterioration, an inhibitory effect on the fluorescent compound formation was observed; however, a definite trend on lipid hydrolysis could not be concluded. Taken together, the results of the present study suggest that the employment of a CA-LA icing system can provide a profitable and practical strategy and be a relevant choice to obtain higher quality, safer chilled fish.

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Figure 1

Anaerobe counts (log CFU g\(^{-1}\) muscle)* in chilled megrim stored under different icing conditions**

* Mean values of three (n=3) replicates. Standard deviations are indicated by bars.

Starting raw fish value: 2.65±0.70.

** Abbreviations of icing conditions as expressed in Table 1.

Figure 2

Enterobacteriaceae counts (log CFU g\(^{-1}\) muscle)* in chilled megrim stored under different icing conditions**

* Mean values of three (n=3) replicates. Standard deviations are indicated by bars.

Starting raw fish value: 1.10±0.20.

** Abbreviations of icing conditions as expressed in Table 1.

Figure 3

Proteolytic bacteria counts (log CFU g\(^{-1}\) muscle)* in chilled megrim stored under different icing conditions**

* Mean values of three (n=3) replicates. Standard deviations are indicated by bars.

Starting raw fish value: 2.10±0.20.

** Abbreviations of icing conditions as expressed in Table 1.
REFERENCES


Quitral, V., Donoso, MªL., Ortiz, J., Herrera, MªV., Araya, H. & Aubourg, S. (2009). Chemical changes during the chilled storage of Chilean jack mackerel...


**TABLE 1**

Aerobe and psychrotroph count assessment (log CFU g⁻¹ muscle)* in chilled megrim stored under different icing conditions**

<table>
<thead>
<tr>
<th>Chilling time (days)</th>
<th>Aerobes</th>
<th>Psychrotrophs</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>C-0</td>
<td>C-125</td>
</tr>
<tr>
<td>0</td>
<td>3.49 a (0.91)</td>
<td>3.49 a (0.91)</td>
</tr>
<tr>
<td>2</td>
<td>4.42 ab (0.38)</td>
<td>4.98 b (0.06)</td>
</tr>
<tr>
<td>6</td>
<td>5.19 b (0.34)</td>
<td>5.37 bc (0.77)</td>
</tr>
<tr>
<td>9</td>
<td>7.89 Bc (0.56)</td>
<td>6.02 Ac (0.09)</td>
</tr>
<tr>
<td>13</td>
<td>8.09 Bc (0.88)</td>
<td>7.05 ABd (0.52)</td>
</tr>
</tbody>
</table>

* Mean values of three replicates (n = 3); standard deviations are indicated in brackets.

For each parameter and for each chilling time, mean values followed by different capital letters (A-C) indicate significant (p<0.05) differences as a result of the icing condition. For each parameter and for each icing condition, values followed by different low-case letters (a-d) denote significant (p<0.05) differences as a result of the chilling time. No letters are indicated when significant differences are not found (p>0.05).

** Abbreviations of icing conditions: C-0 (Control; no acids presence in ice), C-125 (0.125% citric acid and 0.050% lactic acid) and C-175 (0.175% citric acid and 0.050% lactic acid).
TABLE 2

Chemical quality parameters assessment* in chilled megrim stored under different icing conditions**

<table>
<thead>
<tr>
<th>Chilling time (days)</th>
<th>pH</th>
<th>Trimethylamine-N (mg kg(^{-1}) muscle)</th>
<th>Free fatty acids (g kg(^{-1}) lipids)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>C-0</td>
<td>C-125</td>
<td>C-175</td>
</tr>
<tr>
<td></td>
<td>C-0</td>
<td>C-125</td>
<td>C-175</td>
</tr>
<tr>
<td>0</td>
<td>6.41 a</td>
<td>6.41 ab</td>
<td>6.41 ab</td>
</tr>
<tr>
<td></td>
<td>(0.15)</td>
<td>(0.15)</td>
<td>(0.15)</td>
</tr>
<tr>
<td>2</td>
<td>6.58 Ba</td>
<td>6.26 Aa</td>
<td>6.32 Aa</td>
</tr>
<tr>
<td></td>
<td>(0.10)</td>
<td>(0.06)</td>
<td>(0.07)</td>
</tr>
<tr>
<td>6</td>
<td>6.58 a</td>
<td>6.48 b</td>
<td>6.49 b</td>
</tr>
<tr>
<td></td>
<td>(0.07)</td>
<td>(0.06)</td>
<td>(0.06)</td>
</tr>
<tr>
<td>9</td>
<td>6.57 ab</td>
<td>6.39 ab</td>
<td>6.38 ab</td>
</tr>
<tr>
<td></td>
<td>(0.22)</td>
<td>(0.14)</td>
<td>(0.10)</td>
</tr>
<tr>
<td>13</td>
<td>6.84 Bb</td>
<td>6.58 Ab</td>
<td>6.37 Aab</td>
</tr>
<tr>
<td></td>
<td>(0.08)</td>
<td>(0.14)</td>
<td>(0.18)</td>
</tr>
</tbody>
</table>

* Mean values of three replicates (n = 3); standard deviations are indicated in brackets. For each parameter and for each chilling time, mean values followed by different capital letters (A-B) indicate significant (p<0.05) differences as a result of the icing condition. For each parameter and for each icing condition, values followed by different low-case letters (a-d) denote significant (p<0.05) differences as a result of the chilling time. No letters are indicated when significant differences are not found (p>0.05).

** Abbreviations of icing conditions as expressed in Table 1.
<table>
<thead>
<tr>
<th>Chilling time (days)</th>
<th>Peroxide value (meq active oxygen kg(^{-1}) lipids)</th>
<th>Thiobarbituric acid index (mg malondialdehyde kg(^{-1}) muscle)</th>
<th>Fluorescence ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>C-0</td>
<td>C-125</td>
<td>C-175</td>
</tr>
<tr>
<td>0</td>
<td>1.16 a</td>
<td>1.16 a</td>
<td>1.16 a</td>
</tr>
<tr>
<td></td>
<td>(0.43)</td>
<td>(0.43)</td>
<td>(0.43)</td>
</tr>
<tr>
<td>2</td>
<td>2.46 b</td>
<td>1.00 a</td>
<td>1.43 a</td>
</tr>
<tr>
<td></td>
<td>(0.76)</td>
<td>(1.10)</td>
<td>(0.10)</td>
</tr>
<tr>
<td>6</td>
<td>3.48 b</td>
<td>1.68 ab</td>
<td>4.29 b</td>
</tr>
<tr>
<td></td>
<td>(1.68)</td>
<td>(0.93)</td>
<td>(1.40)</td>
</tr>
<tr>
<td>9</td>
<td>4.95 b</td>
<td>2.76 b</td>
<td>6.35 bc</td>
</tr>
<tr>
<td></td>
<td>(2.93)</td>
<td>(0.31)</td>
<td>(2.87)</td>
</tr>
<tr>
<td>13</td>
<td>4.01 b</td>
<td>7.10 c</td>
<td>8.33 c</td>
</tr>
<tr>
<td></td>
<td>(2.30)</td>
<td>(1.49)</td>
<td>(2.02)</td>
</tr>
</tbody>
</table>

* Mean values of three replicates (n = 3); standard deviations are indicated in brackets. For each parameter and for each chilling time, mean values followed by different capital letters (A-B) indicate significant (p<0.05) differences as a result of the icing condition. For each parameter and for each icing condition, values followed by different low-case letters (a-d) denote significant (p<0.05) differences as a result of the chilling time. No letters are indicated when significant differences are not found (p>0.05).

** Abbreviations of icing conditions as expressed in Table 1.
TABLE 4

Evaluation of sensory acceptance* in chilled megrim stored under different icing conditions**

<table>
<thead>
<tr>
<th>Sensory descriptor</th>
<th>Chilling time (days)</th>
<th>Icing conditions</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>C-0</td>
</tr>
<tr>
<td>Skin</td>
<td>2</td>
<td>A</td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>A</td>
</tr>
<tr>
<td></td>
<td>9</td>
<td>A</td>
</tr>
<tr>
<td></td>
<td>13</td>
<td>B</td>
</tr>
<tr>
<td>Eyes</td>
<td>2</td>
<td>A</td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>A</td>
</tr>
<tr>
<td></td>
<td>9</td>
<td>A</td>
</tr>
<tr>
<td></td>
<td>13</td>
<td>B</td>
</tr>
<tr>
<td>External odour</td>
<td>2</td>
<td>A</td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>A</td>
</tr>
<tr>
<td></td>
<td>9</td>
<td>B</td>
</tr>
<tr>
<td></td>
<td>13</td>
<td>C</td>
</tr>
<tr>
<td>Gills</td>
<td>2</td>
<td>A</td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>A</td>
</tr>
<tr>
<td></td>
<td>9</td>
<td>B</td>
</tr>
<tr>
<td></td>
<td>13</td>
<td>B</td>
</tr>
<tr>
<td>Consistency</td>
<td>2</td>
<td>A</td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>A</td>
</tr>
<tr>
<td></td>
<td>9</td>
<td>B</td>
</tr>
<tr>
<td></td>
<td>13</td>
<td>B</td>
</tr>
<tr>
<td>Flesh odour (raw)</td>
<td>2</td>
<td>A</td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>A</td>
</tr>
<tr>
<td></td>
<td>9</td>
<td>B</td>
</tr>
<tr>
<td></td>
<td>13</td>
<td>C</td>
</tr>
<tr>
<td>Flesh odour (cooked)</td>
<td>2</td>
<td>A</td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>A</td>
</tr>
<tr>
<td></td>
<td>9</td>
<td>B</td>
</tr>
<tr>
<td></td>
<td>13</td>
<td>C</td>
</tr>
<tr>
<td>Flesh taste (cooked)</td>
<td>2</td>
<td>A</td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>A</td>
</tr>
<tr>
<td></td>
<td>9</td>
<td>A</td>
</tr>
<tr>
<td></td>
<td>13</td>
<td>C</td>
</tr>
</tbody>
</table>

* Quality categories: E (excellent), A (good), B (fair) and C (unacceptable). Starting fish (day 0) was category E in all descriptors.

** Abbreviations of icing conditions as expressed in Table 1.