Preservative effect of an organic acid-icing system on chilled fish lipids

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SUMMARY

This study provides a first approach concerning a novel chilling strategy, which employs a mixture of different preservative organic acids (ascorbic, citric and lactic) in the icing medium. Thus, ice prepared from water including two different concentrations of a commercial acid mixture-formula (800 ppm and 400 ppm; C-800 and C-400 conditions, respectively) were applied as icing system to three important commercial fish lean species (hake, *Merluccius merluccius*; megrim, *Lepidorhombus whiffiagonis*; angler, *Lophius piscatorius*). Lipid oxidation (peroxide value; thiobarbituric acid index; fluorescent compound formation) and hydrolysis (free fatty acid formation) were evaluated throughout the chilling time (up to 12-15 days) and compared to results obtained in fish kept under traditional ice prepared only from water (C-0 condition); a complementary sensory evaluation was carried out. As a result of employing the C-800 icing condition, a partial inhibition of lipid oxidation and hydrolysis development was obtained that was accompanied by a shelf life enhancement in all cases. Further research taking into account the complementary action of the present organic acids is envisaged. According to the lipid damage analysis undergone, lipid hydrolysis showed to be a more relevant event than lipid oxidation in all fish species tested.

PRACTICAL APPLICATIONS

The actual increasing consumer's demand for high quality fresh products has led to the search for valuable treatments that provide enhancing commercial possibilities. This study provides a first approach to the employment of a novel chilling strategy consisting of an icing system including a mixture of organic acids (ascorbic, citric and lactic), these providing complementary preservative properties (acidulants, antioxidants and antimicrobians). As a result of employing this icing condition, a partial inhibition of lipid oxidation and hydrolysis development was obtained that was accompanied by a shelf life enhancement during the chilled storage of three important lean fish species (hake, megrim and angler). Further studies focused to the optimised employment of these acids by means of this icing strategy are envisaged, taking into account the endogenous antioxidant composition in tested fish species (synergism possibility analysis) and the diffusion rate of organic acids from the icing medium to the fish muscle.

Running Head: Organic acid-icing and chilled fish lipids

Keywords: Fish, chilling, ascorbic, citric, lactic, lipid damage

1. INTRODUCTION

Marine products constitute a highly perishable food group. Deterioration of fish species begins immediately upon capture, and the degree to which it continues depends directly on storage conditions. Flake ice has been the most employed method to cool and store fish products and partially inhibit detrimental effects on the commercial value. However, significant deterioration of sensory quality and nutritional value has been detected in chilled fish as a result of different damage pathways, such as endogenous enzymatic activity, microbial development and lipid oxidation [1, 2].

Assurance of both high quality and safety of chilled seafood is an important actual challenge for fish traders and food technologists. To retard fish damage as long as possible, and accordingly extend shelf life, a wide number of preservative strategies to be combined to flake ice chilling have been tested satisfactorily such as previous chemical and physical treatments [3, 4] and employment of preservative packaging [5, 6].

Among chemical treatments, natural organic acids have shown to represent a relevant choice because of their easy availability, low commercial cost and wide range of permitted concentrations for their use. Thus, ascorbic and citric acids (AA and CA, respectively) and their salts are widely known for their role as chelators, acidulants in biological systems and synergists of primary antioxidants, so that a profitable effect on fish oil and emulsions [7, 8], minced fish [9, 10], fish fillets [11, 12] and whole fish [13] have been observed. Further, lactic acid (LA) has been reported to be effective in suppressing Gram-negative bacteria, which are known to be the most important fish spoiler group; thus, LA pre-treatment has shown to be effective in preserving and extending shelf-life in fish fillets [14, 15] and coated fish [16].

Marine lipids are comprised by highly unsaturated fatty acids that are known to be very prone to oxidation. During fish chilled storage, lipids have been reported to undergo hydrolysis and oxidation reactions that can lead to important losses of sensory and nutritional qualities with an important impact on the commercial value [17, 18]. The present research focuses the lipid damage undergone by three important lean fish species (hake, *Merluccius merluccius*; megrim, *Lepidorhombus whiffiagonis*; angler, *Lophius piscatorius*) during the chilled storage. The study provides a first approach concerning a novel strategy employing ice prepared from an aqueous solution including AA, CA and LA as chilling system. Lipid oxidation and hydrolysis were evaluated throughout the storage time and compared to results obtained in fish kept under traditional ice only prepared from water. In a previous experiment [19], ice including polyphenolic compounds from rosemary and oregano aqueous extracts was applied to a fatty fish species (Chilean jack mackerel; *Trachurus murphy*); the employment of such icing system as chilling medium led to a partial inhibition of lipid oxidation.

2. MATERIALS AND METHODS

2.1. Preparation of icing systems including organic acids

A commercial formula (BPS2) including an organic acid-mixture was supplied by ATALANTA S.L. (Vigo, Spain) for employment in the present research. Such a product consists of a water-soluble viscous liquid including AA, CA and LA (1 meq acid/ 120 mg product) in glycerol, being regarded as safe (GRAS) for use in foods according to European and American administrations [20, 21].

Preliminary trials were carried out in order to assess the most convenient product concentration to be included in the icing system. For it, the effect on sensory acceptance (appearance, texture, gills, odour and colour) of a wide range (70-2000 ppm) of aqueous concentrations of the organic acid-mixture was checked. As a result, 800 ppm and 400 ppm concentrations showed to provide the most convenient results. Accordingly, both concentrations were chosen to be employed in the present research (C-800 and C-400 conditions, respectively) and be compared to fish kept under traditional ice prepared from water (C-0 condition).

2.2. Raw fish, processing and sampling

Fresh hake (*Merluccius merluccius*; 96 individuals), megrim (*Lepidorhombus whiffiagonis*; 78 individuals) and angler (*Lophius piscatorius*; 78 individuals) were caught near the Galician Atlantic coast (North-western Spain) in Autumn 2009 and transported on ice to the laboratory. The length and weight of the fish specimens was included in the following ranges: 32-34 cm and 200-300 g (hake), 19-23 cm and 90-120 g (megrim) and 24-27 cm and 275-350 g (angler).

Upon arrival in the laboratory, six individual fishes of each species were separated and considered as starting raw fish (day 0); for it, three (n=3) different groups (2 individuals per group) were considered for each species and analysed independently. The remaining fish were divided into three batches (30 individuals in each batch for hake; 24 individuals per batch for both megrim and angler), placed in boxes and directly surrounded by different kinds of ice (C-800, C-400 and C-0 conditions, respectively), according to the results obtained in the above mentioned preliminary trials.

In each case, the fish individuals were surrounded by ice at a 1:1 fish-to-ice ratio. All batches were placed in a refrigerated room (4 ºC). Boxes employed allowed draining

and ice was renewed when required. Fish samples from the different icing conditions were taken for analysis on days 1, 5, 8 and 12; day 15 was also considered in the case of hake. At each sampling point, six individuals of each species per batch were taken for analysis, so that three (n=3) independent groups (two individuals per group) were considered for each batch and species.

2.3. Composition analyses

Moisture content was determined by the difference between the weight of fresh homogenised white muscle (1-2 g) and the weight recorded after 4 h at 105 ºC. Results were expressed as g water/ 100 g muscle.

Lipids were extracted from the fish white muscle by the Bligh and Dyer [22] method, by employing a single-phase solubilisation of the lipids using a chloroform-methanol (1:1) mixture. Quantification results were expressed as g lipid/ 100 g muscle.

2.4. Lipid oxidation measurement

The peroxide value (PV) was determined in the lipid extract by peroxide reduction with ferric thiocyanate, according to the Chapman and McKay [23] method. Results were expressed as meq active oxygen/ kg lipids.

The thiobarbituric acid index (TBA-i) was determined according to Vyncke [24]. This method is based on the reaction between a trichloracetic acid extract of the fish muscle and thiobarbituric acid. Content on thiobarbituric acid reactive substances (TBARS) was spectrophotometrically measured at 532 nm and results were expressed as mg malondialdehyde/ kg muscle.

Formation of fluorescent compounds was determined by measurements at 393/463 nm and 327/415 nm as described by Aubourg et al. [25]. The relative fluorescence (RF) was

calculated as follows: RF = 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 in 0.05 M H_2SO_4) at the corresponding wavelength. The fluorescence ratio (FR) was calculated as the ratio between the two RF values: FR = $RF_{393/463\ nm}$ / $RF_{327/415\ nm}$. The FR value was determined in the aqueous phase resulting from the lipid extraction of the fish muscle [22].

2.5. Lipid hydrolysis assessment

Free fatty acid (FFA) content was determined in the lipid extract of the fish muscle by the Lowry and Tinsley [26] method based on complex formation with cupric acetate-pyridine followed by spectrophotometric (715 nm) assessment. Results were expressed as g FFA/ 100 g lipids.

2.6. Sensory analysis

Sensory analysis was conducted by a sensory panel consisting of five experienced judges, according to guidelines concerning fresh and refrigerated fish [27]. Panellists had been involved in sensory analysis of different kinds of fish foods during the last 10 years. Previously to the present experiment, a special training was carried out on chilled hake, megrim and angler.

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: eyes, gills, external odour, muscle odour (raw and cooked fish) and taste (cooked fish). At each sampling time, the fish muscle portions were presented to panellists in individual trays and were scored individually. The panel members shared samples tested.

2.7. Statistical analysis

Data (n = 3) obtained from the different chemical analyses were subjected to the ANOVA method ($p<0.05$) to explore differences by two different ways: icing conditions effect and chilling time effect (Statsoft, Statistica, version 6.0, 2001); comparison of means was performed using a least-squares difference (LSD) method. Correlation analysis among parameters (chilling time, lipid damage indices and sensory acceptance) was also carried out. In them, linear fittings are expressed; otherwise, the kind of fitting is mentioned.

3. RESULTS AND DISCUSSION

3.1. Moisture and lipid contents

Moisture contents obtained for the three species are expressed in Table 1. Values included in the 79-81 g/ 100 g muscle range were observed in starting raw fish, which agrees to results previously obtained in the same species and related lean fish species [25, 28-31]. Comparison among fish individuals from the different icing conditions tested provided some differences; however, a clear pattern could not be concluded, so that no effect ($p>0.05$) of the organic acid presence in ice could be inferred on water content in fish muscle. Meantime, an increasing ($p<0.05$) moisture value could be observed in all kinds of samples with chilling time; thus, good correlation values were obtained between both parameters (chilling time and moisture content) for hake (r^2 = 0.93-0.94, logarithmic fitting) and megrim (r^2 = 0.91-0.94), being fair in the case of angler (r^2 = 0.83-0.93, logarithmic fitting). This increase can be explained as a result of

contact with the ice medium and has already been described in previous research related to lean fish species [25].

Concerning the lipid content of fish muscle, hake and megrim provided a higher value range (0.55-0.70 g/ 100 g muscle) than angler (0.40-0.45 g/ 100 g muscle). This result agrees to the fact that a higher ($p<0.05$) moisture content was observed for this latest species, according to a known inverse ratio between both (water and lipids) constituent proportions [28]. Mean values obtained for lipid content showed some decreasing tendency with icing time; however, differences were not found significant ($p>0.05$) as a result of marked fish-to-fish differences.

3.2. Lipid oxidation assessment

Lipid oxidation was studied by means of different quality indices corresponding to different steps included in the fish lipid oxidation development.

Related to hake chilled storage (Table 2), C-800 condition led in most cases to individual fishes showing lower mean values for peroxides, TBARS and fluorescence development than their corresponding fish samples from C-400 and C-0 conditions; when compared to control fish, such differences were found significant in most cases, so that an inhibitory effect on lipid oxidation development could be inferred for the organic acid presence in ice at the highest concentration. Concerning C-400 condition, an inhibitory effect could also be concluded according to the FR assessment (days 1, 12 and 15).

Taking into account the peroxide (lower scores than 5.0 in all cases) and the TBARS (lower scores than 0.9 in all cases) values, hake lipid oxidation can be considered low in all kinds of fish samples, according to previous research concerning chilled storage of this species [29, 30]. In general terms, increasing values could be observed with time in

all kinds of samples for the different quality indices. However, in the case of PV and TBA-i, some decreases in advanced chilled stages (days 12 and 15) could also be observed; such decreases can be explained on the basis of the high reactivity of molecules to be measured under both quality indices [25, 32]. As a result, the best correlation values between chilling time and lipid oxidation development could be observed when considering the FR assessment (r^2 = 0.82-0.87), also in agreement to previous research [30].

Related to megrim (Table 3), an inhibitory effect of both mixture concentrations (C-400 and C-800 conditions) could be observed on fluorescent compound formation in the 8-12-day period. However, peroxide and TBARS formation did not provide a definite tendency during the 0-8-day period; at the end of the experiment, higher values were found for fish kept under C-400 condition.

In this case (megrim fish), lipid oxidation development can also be considered low according to the peroxide development (scores below 7.5 in all cases) and TBARS formation (scores below 1.0 in all cases). Chilling time led in most cases to increases for the PV and TBA-i determinations; however, some decreasing values were produced in advanced chilled stages (day 12), that can be explained again as a result of breakdown or interaction of molecules susceptible to be measured under such indices. As with hake, FR assessment provided in all icing systems a gradual increase so that a good correlation value could be found between chilling time and FR parameter (r^2 = 0.92-0.93); this quality index already showed to be accurate during a previous chilled storage experiment of megrim [33].

For chilled angler (Table 4), a lower ($p<0.05$) peroxide formation could be observed in individuals kept under C-800 condition when compared to their counterparts under control conditions; such a conclusion is also valid in most cases for C-400 condition

fish. However, this inhibitory effect could not be observed for the two other indices (TBA-i and FR), which did not provide a definite effect ($p>0.05$) for the organic acid presence in ice during the chilled storage of angler.

Present results conclude that lipid oxidation development has shown to be specially low in angler fish. Peroxide scores remained in all cases under 4.0 value, while TBARS formation showed in all cases scores under 0.20 value. Additionally, peroxide value showed a general decrease at advanced stages (day 12) of chilled storage. It is inferred that lipid oxidation development is not a relevant damage mechanism in this species under chilling conditions. This conclusion agrees to the above mentioned low lipid content (0.40-0.45 g/ 100 g muscle). In spite of the low primary and secondary lipid oxidation development, FR assessment showed an increasing tendency in fish samples corresponding to all icing conditions; as a result, a fair correlation value was obtained between chilling time and FR (r^2 = 0.83-0.89, quadratic fitting).

The partial inhibition of oxidation development found in the present research for the three species as a result of the organic acid presence in ice agrees to previous research where AA and CA have shown a profitable antioxidant effect when applied as a preliminary treatment. Thus, CA and its salts have shown to play a synergist role with primary antioxidants and oxygen scavengers during the refrigerated [10, 34] and frozen [12, 13] storage of marine species. In the same way, AA and its salts have been reported to act as antioxidants in fish oil [7], minced fish [9], fish fillets [11] and whole fish [13] because of their oxygen scavenger and reducing roles.

Lipid oxidation development has been recognised as a complex process where different kinds of molecules are produced, most of them unstable, susceptible to breakdown and originate lower weight compounds, or react with other molecules (nucleophilic-type, mostly) present in the fish muscle; as a result, determination of each kind of compound

cannot always provide an accurate method for the quality loss assessment in fish. In the present research, all species showed a low primary and secondary lipid oxidation compound formation, that was accompanied by some content decreases in advanced chilled stages. The electrophilic character of such lipid oxidation compounds has lead them to interact with food constituents possessing nucleophilic functions [25, 32], so that FR assessment (tertiary lipid oxidation compounds) has provided the best correlation values with chilling time in all three species tested.

3.3. Lipid hydrolysis development

Although lipid hydrolysis has been shown to occur during fish chilled storage, the formation of FFA itself does not lead to nutritional losses. However, accumulation of FFA has been related to some extent to lack of acceptability, because FFA are known to have detrimental effects on protein properties [35] and oxidise faster than higher molecular weight lipid classes (namely, triglycerides and phospholipids) by providing a greater accessibility (lower steric hindrance) to oxygen and other pro-oxidant molecules [36]. In addition, previous research has shown FFA assessment to be an accurate tool for assessing freshness loss during lean fish chilling [37]. According to this, examining the extent of lipid hydrolysis was deemed important to the present research.

FFA formation showed a lower ($p<0.05$) formation in hake fish kept under C-800 condition when compared to its counterpart corresponding to C-400 and C-0 conditions (Fig. 1). However, no inhibitory effect ($p>0.05$) could be observed as a result of employing ice including a 400 ppm concentration of the organic acid-mixture. As in previous research [30], lipid hydrolysis development in chilled hake provided an increasing tendency with chilling time in all icing conditions, so that a good correlation value was obtained between both parameters (r^2 = 0.93-0.95, quadratic fitting).

A similar result could be concluded for megrim fish (Fig. 2). Thus, a lower ($p<0.05$) FFA formation could be observed in the 8-12-day period for individual fishes corresponding to C-800 condition when compared to their counterpart samples from C-400 and C-0 conditions. Again, no effect could be attributed to the C-400 condition, as no differences ($p>0.05$) could be inferred with fish samples corresponding to control. An important FFA formation was observed in chilled megrim under any of the icing conditions studied, so that a good correlation value was obtained between both parameters (chilling time and FFA content) for the different icing conditions (r^2 = 0.93-0.94, quadratic fitting). A marked lipid hydrolytic activity had already been shown during a previous chilled experiment of megrim [33].

Concerning angler fish (Fig. 3), a lower ($p<0.05$) hydrolysis development could be observed for fish corresponding to the C-800 condition when compared to fish kept under the two other icing conditions. As for hake and megrim, C-400 condition did not provide ($p>0.05$) an inhibitory effect on FFA formation. A progressive formation of FFA was produced with chilling time in all cases; thus, good correlation values were obtained between chilling time and FFA content (r^2 = 0.91-0.92).

FFA formation during chilling storage has been reported to be produced as a result of endogenous enzyme activity and microbial activity [1, 17]. Before the end of the microbial lag phase (up to 6-9 days, depending on several factors), FFA formation has been reported to be produced mostly as a result of endogenous enzyme (namely, lipases and phospholipases) activity. Later on, microbial activity should gain importance, so that FFA formation is then mostly produced as a result of bacterial catabolic processes. According to this profile, present results on FFA formation in hake and megrim show a first stage of low FFA formation (0-8 days and 0-5 days, respectively) corresponding mostly to the endogenous enzymatic activity. Then, a marked increase is produced (a

quadratic fitting has been mentioned above for both species) that could be explained as a result of the microbial activity development. In the case of angler, a marked difference between both FFA formation phases is not apparent, so that a progressive FFA content increase is observed (a linear fitting has been mentioned above) throughout the whole experiment.

Concerning the effect of the organic acid presence in the icing system, a microbial activity inhibition during the second phase of the chilling time has been observed in the three species tested. To our knowledge, this preservative effect is described for the first time during the chilled storage of lean fish species. As being molecules supporting acidulant properties, all three acids can be found responsible for this microbial inhibition [1, 4]. However, this effect has been reported to be specially important in the case of LA, as being recognised as effective in suppressing Gram-negative bacteria activity, which are known to be the most important fish spoiler group. Thus, previous research reports on an extension of shelf life in fish fillets [14, 15] and coated fish [16] by employing LA as a previous treatment.

Good correlation values were obtained between the FFA content and the FR value for hake (r^2 = 0.91-0.93) and megrim (r^2 = 0.89-0.98). Such a result has already been observed in previous research in chilled lean fish species [25, 30] and proves a strong connexion between lipid hydrolysis and oxidation. In this sense, a FFA pro-oxidant effect has been explained on the basis of a catalytic effect of the carboxyl group on the formation of free radicals by the decomposition of hydroperoxides [38]. On the other hand, present research shows a poor correlation value between both parameters (FR and FFA content) in the case of angler (r^2 = 0.75-0.89). This result can be explained on the basis that a very low lipid oxidation development has been produced in this species during the present research.

3.4. Sensory evaluation

Sensory evaluation was carried out according to parameters expressed in the experimental section (Table 5). Under all icing conditions, a quality decrease was observed as a result of increasing the chilling time. An extended shelf life time was obtained for fish kept in ice including the highest organic acid content when compared to its counterpart belonging to C-400 and C-0 conditions. Under such C-800 condition, hake, megrim and angler were still acceptable after 12, 8 and 12 days of chilled storage, respectively. No profitable effect of C-400 condition on sensory acceptance could be observed when compared to control fish.

Among the different kinds of molecules produced as a result of lipid damage, secondary lipid oxidation compounds are considered the chief compounds responsible for oxidised flavours [39]. Present research provided poor correlation values for sensory acceptance with the TBA-i. This result can be explained on the basis that lipid oxidation development was relatively low, so that a negligible rancid odour formation was produced, according to previous research concerning hake chilling [29, 30]. Previous research has shown a strong incidence of FFA accumulation on fish quality loss [40], being the FFA presence associated in some extent to the lack of sensory acceptability and strongly interrelated with off-odour development [41]. However, poor correlation values were obtained in the present research between sensory acceptance and FFA content, this result being explained again on the basis of the negligible rancid odour formation.

4. CONCLUDING REMARKS

The actual increasing consumer's demand for high quality fresh products has led to the search for valuable and practical treatments that may provide enhancing commercial possibilities. With this basic objective, the present study provides a first approach to the employment of a novel strategy consisting of an icing system including a mixture of organic acids (AA, CA and LA), these providing complementary preservative properties (acidulants, antioxidants and antimicrobians). According to the lipid damage (hydrolysis and oxidation) development in the lean fish species tested, results obtained showed a positive role of the organic acid-mixture present in the icing medium, so that a partial inhibition of lipid oxidation and hydrolysis development was attained. This icing strategy was already tested by employing ice prepared from aqueous solutions of plant extracts including polyphenolic compounds [19]; in such experiment, a partial damage inhibition was produced during the chilled storage of a fatty fish species (Chilean jack mackerel; *Trachurus murphy*).

Further studies focused to the positive role of this icing strategy are envisaged. A great effort ought to be addressed towards knowledge of the organic acid range where optimal effects could be attained [42], this including knowledge of the endogenous antioxidant composition corresponding to the fish species to be tested (synergism possibility analysis) and towards research checking the diffusion rate of organic acids from the icing medium to the fish muscle.

Although most research has been previously reported for quality changes in chilled Atlantic hake [29, 43, 44], studies related to the lipid fraction have been scarce and incomplete [30, 31, 45]. In addition, research related to the lipid fraction damage during the chilled storage of megrim [33] and angler [31] is even more scarce and incomplete.

According to the results obtained in the present research, lipid hydrolysis showed to be a more relevant event than lipid oxidation in all lean fish species tested.

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Conflict of interest statement

The authors have declared no conflict of interest.

FIGURE LEGENDS

Figure 1: Evolution of free fatty acid (FFA) formation [g/ 100 g lipids]* in chilled hake stored under different icing conditions**

* Mean values of three (n=3) independent determinations are given. Standard deviations are denoted by bars. Starting raw fish value: 0.99±0.17.

** Icing conditions as expressed in Table 1.

Figure 2: Evolution of free fatty acid (FFA) formation [g/ 100 g lipids]* in chilled megrim stored under different icing conditions**

* Mean values of three (n=3) independent determinations are given. Standard deviations are denoted by bars. Starting raw fish value: 0.06±0.05.

** Icing conditions as expressed in Table 1.

Figure 3: Evolution of free fatty acid (FFA) formation [g/ 100 g lipids]* in chilled angler stored under different icing conditions**

* Mean values of three (n=3) independent determinations are given. Standard deviations are denoted by bars. Starting raw fish value: 1.11±0.42.

** Icing conditions as expressed in Table 1.

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TABLE 1: Evolution of moisture content [g/ 100 g muscle]* in chilled fish species stored under different icing conditions**

Fish species	Icing condition	Chilling storage time [days]					
		0	1	5	8	12	15
Hake	C-0	79.8 (0.2)	80.8 (0.3)	82.0 y (0.1)	82.0 (0.1)	82.7 (0.4)	83.0 (0.8)
	C-400		80.7 (0.1)	81.4 yx (0.5)	82.1 (0.3)	82.2 (0.4)	82.9 (0.3
	C-800		80.2 (0.4)	81.2 z (0.2)	82.2 (0.4)	82.5 (0.2)	82.7 (0.3)
Megrim	C-0	79.0 (0.3)	78.4 (0.1)	80.7 (0.2)	81.3 (0.5)	82.2 (0.2)	–
	C-400		78.9 (0.4)	80.4 (0.4)	81.0 (0.5)	82.0 (0.2)	–
	C-800		78.8 (0.2)	80.4 (0.4)	80.9 (0.1)	81.5 (0.4)	–
Angler	C-0	81.0 (0.3)	83.6 y (0.8)	83.6 (0.2)	84.3 y (0.2)	84.2 (0.5)	–
	C-400		82.2 z (0.1)	84.0 (0.8)	84.6 y (0.4)	84.5 (0.5)	–
	C-800		82.8 y (0.3)	84.0 (0.5)	83.5 z (0.4)	84.5 (0.4)	–

* Mean values of three (n = 3) independent determinations are expressed. Standard deviations are indicated in brackets. For each species and for each chilling time, mean values followed by different letters (z, y, x) denote significant ($p<0.05$) differences.

** Icing conditions: C-800 and C-400 (ice prepared from an aqueous solution including 800 ppm and 400 ppm of the organic acid-formula, respectively) and C-0 (ice prepared from water; control).

TABLE 2

Evolution of lipid oxidation* in chilled hake stored under different icing conditions**

Chilling storage time [days]	Lipid oxidation assessment								
	Peroxide value [meq active oxygen/ kg lipids]			Thiobarbituric acid index [mg malondialdehyde/ kg muscle]			Fluorescence ratio		
	C-0	C-400	C-800	C-0	C-400	C-800	C-0	C-400	C-800
0	0.66 (0.37)			0.22 (0.10)			0.20 (0.04)		
1	0.94 (0.26)	0.76 (0.43)	0.68 (0.51)	0.38 y (0.08)	0.16 z (0.07)	0.17 z (0.04)	0.38 y (0.02)	0.26 z (0.05)	0.35 yz (0.05)
5	2.92 x (0.77)	1.59 y (0.29)	0.95 z (0.21)	0.36 y (0.11)	0.20 yz (0.07)	0.19 z (0.04)	0.15 (0.04)	0.14 (0.05)	0.17 (0.05)
8	2.53 yz (0.58)	3.54 y (0.51)	2.74 z (0.21)	0.53 (0.32)	0.55 (0.25)	0.44 (0.07)	0.28 (0.16)	0.22 (0.13)	0.15 (0.06)
12	3.85 yz (1.06)	4.98 y (0.35)	3.44 z (0.17)	0.82 y (0.42)	0.79 y (0.17)	0.32 z (0.18)	0.94 y (0.17)	0.55 z (0.15)	0.44 z (0.09)
15	4.72 y (1.17)	4.01 yz (1.01)	2.86 z (0.49)	0.43 y (0.06)	0.29 z (0.05)	0.27 z (0.08)	2.65 y (0.43)	1.13 z (0.36)	0.80 z (0.16)

* Mean values of three (n = 3) independent determinations are expressed. Standard deviations are indicated in brackets. For each parameter and for each chilling time, mean values followed by different letters (z, y, x) denote significant ($p<0.05$) differences.

** Icing conditions (C-0, C-400 and C-800) as expressed in Table 1.

TABLE 3

Evolution of lipid oxidation* in chilled megrim stored under different icing conditions**

Chilling storage time [days]	Lipid oxidation assessment								
	Peroxide value [meq active oxygen/ kg lipids]			Thiobarbituric acid index [mg malondialdehyde/ kg muscle]			Fluorescence ratio		
	C-0	C-400	C-800	C-0	C-400	C-800	C-0	C-400	C-800
0	2.79 (1.12)			0.21 (0.05)			0.08 (0.20)		
1	2.33 (0.79)	2.04 (0.55)	2.19 (0.21)	0.29 y (0.07)	0.16 z (0.04)	0.22 yz (0.15)	0.90 (0.15)	0.75 (0.02)	0.83 (0.09)
5	2.68 (0.99)	2.79 (0.25)	1.89 (0.64)	0.31 (0.08)	0.44 (0.09)	0.45 (0.10)	1.85 (0.41)	1.76 (0.35)	1.91 (0.36)
8	5.78 (0.49)	4.76 (0.21)	3.37 (1.05)	0.62 (0.19)	0.50 (0.16)	0.48 (0.09)	3.62 y (0.23)	2.03 z (0.48)	2.22 z (0.38)
12	3.57 z (0.73)	7.03 y (0.57)	4.58 z (0.41)	0.37 z (0.08)	0.96 y (0.03)	0.44 z (0.11)	7.09 y (1.35)	3.88 z (1.14)	2.99 z (0.53)

* Mean values of three (n = 3) independent determinations are expressed. Standard deviations are indicated in brackets. For each parameter and for each chilling time, mean values followed by different letters (z, y) denote significant ($p<0.05$) differences.

** Icing conditions (C-0, C-400 and C-800) as expressed in Table 1.

TABLE 4

Evolution of lipid oxidation* in chilled angler stored under different icing conditions**

Chilling storage time [days]	Lipid oxidation assessment								
	Peroxide value [meq active oxygen/ kg lipids]			Thiobarbituric acid index [mg malondialdehyde/ kg muscle]			Fluorescence ratio		
	C-0	C-400	C-800	C-0	C-400	C-800	C-0	C-400	C-800
0		1.49 (0.57)			0.10 (0.08)			0.37 (0.09)	
1	2.48 (0.40)	2.09 (0.50)	2.35 (0.19)	0.06 (0.01)	0.08 (0.01)	0.14 (0.08)	0.35 z (0.10)	0.31 z (0.10)	0.55 y (0.03)
5	2.81 (0.24)	2.44 (0.48)	2.63 (0.36)	0.11 (0.03)	0.10 (0.03)	0.08 (0.03)	0.38 (0.06)	0.45 (0.08)	0.43 (0.04)
8	3.94 yz (0.62)	4.81 y (0.86)	2.97 z (0.70)	0.09 (0.02)	0.16 (0.08)	0.12 (0.08)	0.80 (0.09)	0.78 (0.07)	0.71 (0.30)
12	3.51 y (0.21)	2.60 yz (0.80)	2.39 z (0.54)	0.03 z (0.01)	0.12 y (0.05)	0.07 yz (0.04)	0.93 (0.25)	1.13 (0.31)	0.80 (0.31)

* Mean values of three (n = 3) independent determinations are expressed. Standard deviations are indicated in brackets. For each parameter and for each chilling time, mean values followed by different letters (z, y) denote significant ($p<0.05$) differences.

** Icing conditions (C-0, C-400 and C-800) as expressed in Table 1.

TABLE 5

Evolution of sensory quality* in chilled fish species stored under different icing conditions**

Fish species	Icing condition	Chilling storage time [days]					
		0	1	5	8	12	15
Hake	C-0	E	A	B	B	C	C
	C-400	E	A	A	B	C	C
	C-800	E	A	A	B	B	C
Megrim	C-0	A	A	B	C	C	–
	C-400	A	A	B	C	C	–
	C-800	A	A	B	B	C	–
Angler	C-0	A	A	B	B	C	–
	C-400	A	A	B	B	C	–
	C-800	A	A	A	A	B	–

* Quality categories: E (highest), A (good), B (fair) and C (unacceptable).

** Icing conditions as expressed in Table 1.

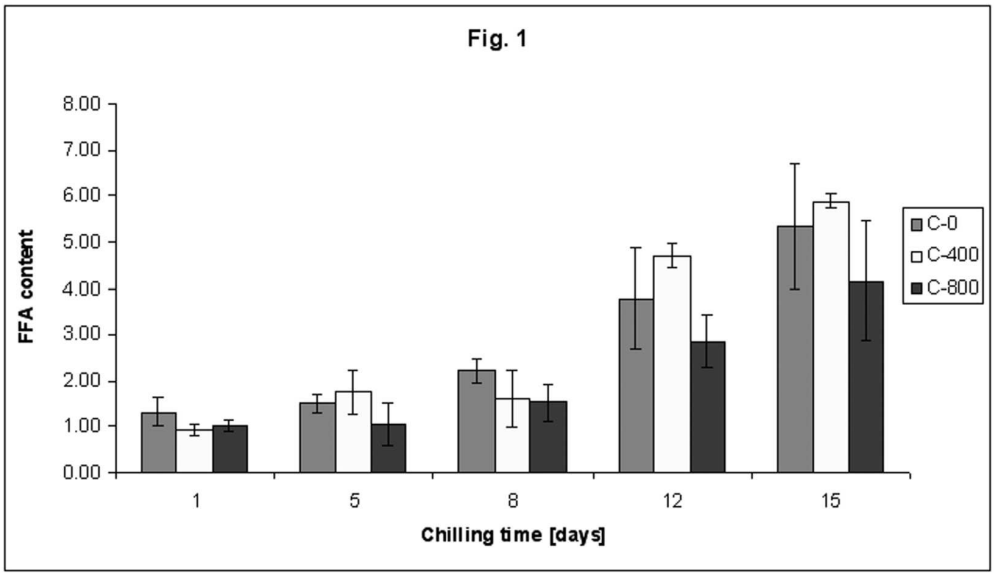

Figure 1
69x40mm (600 x 600 DPI)

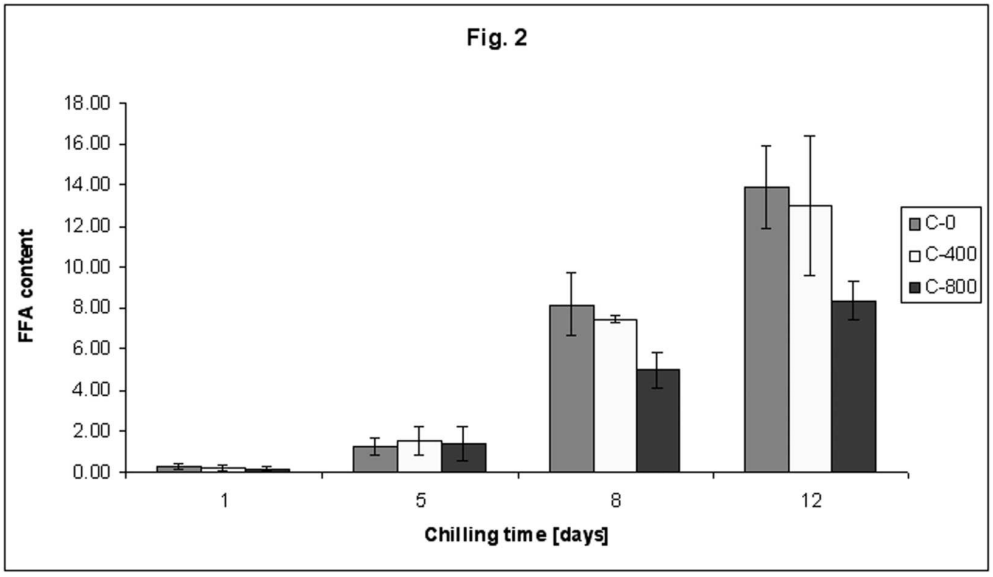

Figure 2
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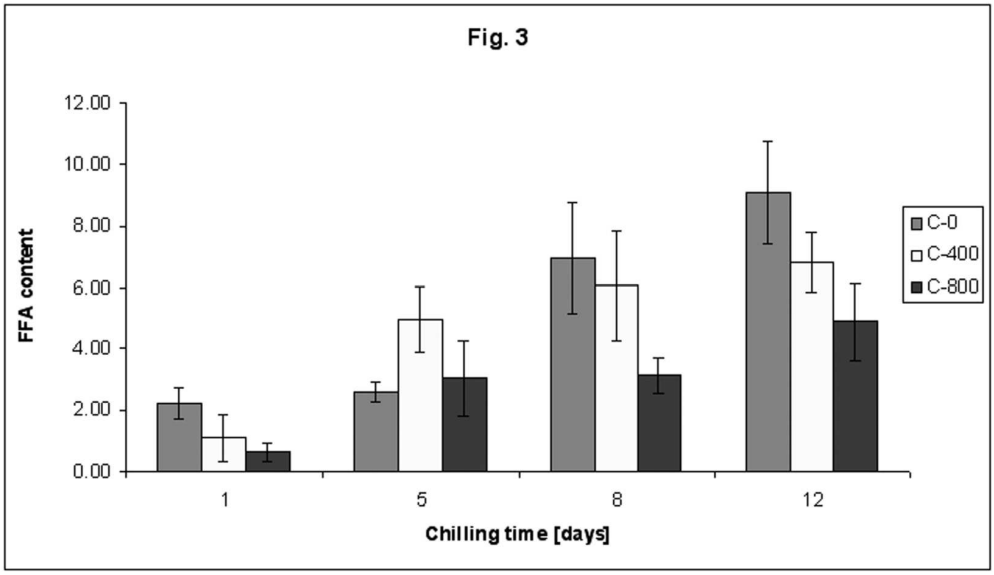

Figure 3
69x40mm (600 x 600 DPI)