Effect of a two-step natural organic acid-treatment on microbial activity and lipid damage during blue whiting (*Micromesistius poutassou*) chilling

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SUMMARY

A novel approach was carried out to inhibit quality loss in chilled blue whiting (*Micromesistius poutassou*). For it, a natural organic acid-mixture including ascorbic, citric and lactic acids was applied in a two-step processing. First, as an aqueous dipping medium previous to chilling storage; then, present in ice employed as chilling system. According to indices related to microbiological activity (namely, aerobe and psychrotroph counts) and lipid damage development, results obtained showed a profitable role of the organic acid-mixture; thus, a partial inhibition of both damage pathways was attained, this leading to an increased shelf life time of chilled blue whiting when compared to control fish. Lipid hydrolysis (free fatty acid formation) showed to be a more relevant event than lipid oxidation (peroxide and thiobarbituric acid reactive substance formation) in chilled blue whiting. Microbiological group counts remained as acceptable values for all kinds of samples throughout a 9-day chilling study.

Running Head: Natural organic acid and blue whiting chilling

Keywords: *Micromesistius poutassou*, ascorbic, citric, lactic, dipping, icing, microbial activity, lipid damage

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1. INTRODUCTION

Deterioration of marine species begins immediately upon capture or harvest, 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 (Whittle et al., 1990; Olafsdóttir et al., 1997). 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 (Toledo-Flores & Zall, 1992; Ashie et al., 1996) and employment of preservative packaging (Ozen & Floros, 2001; Sivertsvik et al., 2002).

Among previous chemical treatments to chilling storage, 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) are widely known for their role as chelators, acidulants in biological systems and synergists of primary antioxidants, so that a profitable effect on minced fish (Hwang & Regenstein, 1988; Stodolnik et al., 1992), fish fillets (Badii & Howell, 2002; Kilinc et al., 2009; Pourashouri et al., 2009) and whole fish (Aubourg et al., 2004) has 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 (Kim et al., 1995; Metin et al., 2001; Erkan, 2003) and coated fish (Gogus et al., 2006).

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Blue whiting (*Micromesistius poutassou*) is a gadoid fish abundant in the northeast Atlantic. Previous research has shown its profitable nutritional support to human diet (Dagbjartsson, 1975; Martínez-Valverde et al., 2000), being most efforts focused to its employment in the manufacture of restructured products (Montero et al., 1998; Pérez-Mateos et al., 2002). However, since it is also directly consumed, blue whiting suitability to freezing (Huidobro & Tejada, 1995; Aubourg et al., 2007), cold-smoking (Gómez-Guillén et al., 2009) and canning (Oehlenschläger et al., 2008) processes has also been tested. Concerning its chilled storage, previous research accounts for the nucleotide degradation and volatile amine formation (Smith et al., 1980), lipid oxidation development (Aubourg et al., 1998) and trimethylamine oxide breakdown into formaldehyde and dimethylamine (Rey-Mansilla et al., 1999).

In the present research, a novel approach is carried out to inhibit quality loss in chilled blue whiting. For it, a natural organic acid-mixture including AA, CA and LA was applied in a two-step processing. First, as an aqueous dipping medium previously to chilling storage; then, present in the ice employed as chilling system. Microbial activity and lipid damage development were evaluated throughout the storage time and compared to control fish.

2. MATERIALS AND METHODS

2.1. The natural organic acid-mixture and preliminary trials

A commercial formula (BPS2) including a natural organic acid-mixture was supplied by *Atlantic one, 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 (Madrid et al., 1994; Giese, 1996).

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Preliminary trials were carried out in order to assess the most convenient product concentration to be included both in the dipping medium as well as in the icing system. For it, the effect on sensory acceptance (appearance, texture, gills, odour and colour) of a wide concentration range (70-2000 ppm) of the organic acid-mixture was checked. As a result, 800 ppm concentration showed to provide the most convenient results when employed in both processing steps. Accordingly, this concentration was chosen for being employed in the present research.

2.2. Raw fish, processing and sampling

Fresh blue whiting (72 individuals) were caught near the Galician Atlantic coast (northwestern Spain) in Winter 2010 and transported on ice to the laboratory. The length and weight of the fish specimens were included in the following ranges: 22-25 cm and 70-120 g, respectively.

Upon arrival in the laboratory, eight individual fishes were separated and considered as starting raw fish (day 0); for it, two different groups (4 individuals per group) were considered and analysed independently. The remaining fish were divided into two batches (32 individuals in each batch). The first batch (preserved fish; P batch) was dipped in a 800 ppm aqueous solution of the organic acid-mixture during 2 minutes (ca. 290 g fish L^{-1} solution); after that, the fish were placed in boxes and directly surrounded by ice prepared with a 800 ppm aqueous solution of the organic acid-mixture.

Fish corresponding to the second batch (control fish; C batch) was dipped in water during 2 minutes (ca. 290 g fish L^{-1} solution) and then placed in boxes surrounded by traditional ice prepared with water.

In both batches, the fish individuals were surrounded by ice (with or without organic acid-mixture, respectively) at a 1:1 fish-to-ice ratio. Both batches were placed in a

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refrigerated room (4 ºC). Boxes employed allowed draining and ice was renewed when required. Fish samples from the two different batches were taken for analysis on days 2, 5, 7 and 9. At each sampling point, eight individuals of each batch were taken for analysis, being considered into two groups (four individuals in each group) that were studied independently.

2.3. Microbial activity measurement

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 a stomacher (AES, Combourg, France) as previously described (Ben-Gigirey et al., 1998, 1999).

In all cases, serial dilutions from the microbial extracts were prepared in 0.1% peptone water. Total aerobes were investigated by surface inoculation in plate count agar (PCA, Oxoid Ltd., London, UK), after incubation at 30 ºC for 48 h. Psychrophiles were also investigated in PCA but incubation was carried out at 7-8 ºC for 7 days. *Enterobacteriaceae* were investigated by pour platting on Violet Red Bile Agar (VRBA) (Merck, Darmstadt, Germany) after incubation at 37 ºC for 24 h. Microorganisms exhibiting a proteolytic or lipolytic phenotype were investigated in casein-agar medium or tributyrin-agar, respectively, after incubation at 30 ºC for 48 h, as previously described (Ben-Gigirey et al., 2000).

In all cases, bacterial counts were transformed into log CFU g^{-1} muscle before undergoing statistical analysis.

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1 **2.4. Proximate composition and pH assessment**

2 Moisture content was determined by the difference between the weight of fresh
3 homogenised white muscle (1-2 g) and the weight recorded after 4 h at 105 ºC. Results
4 were calculated as g water kg^{-1} muscle.
5 Lipids were extracted from the fish white muscle by the Bligh and Dyer (1959) method,
6 by employing a single-phase solubilisation of the lipids using a chloroform-methanol
7 (1:1) mixture. Quantification results were calculated as g lipid kg^{-1} muscle.
8 The evolution of pH values in fish muscle along storage time was determined by means
9 of a 6-mm diameter insertion electrode (Crison, Barcelona, Spain).
10

11 **2.5. Lipid damage assessment**

12 The peroxide value (PV) was determined spectrophotometrically (Beckman Coulter,
13 DU 640, London, UK) in the lipid extract by previous peroxide reduction with ferric
14 thiocyanate, according to the Chapman and McKay (1949) method. Results were
15 expressed as meq active oxygen kg^{-1} lipids.
16 The thiobarbituric acid (TBA) index was determined according to Vyncke (1970). This
17 method is based on the reaction between a trichloracetic acid extract of the fish muscle
18 and TBA. Content on thiobarbituric acid reactive substances (TBARS) was
19 spectrophotometrically measured at 532 nm and results were expressed as mg
20 malondialdehyde kg^{-1} muscle.
21 Formation of fluorescent compounds (Fluorimeter LS 45; Perkin Elmer España, Tres
22 Cantos, Madrid, Spain) was determined by measurements at 393/463 nm and 327/415
23 nm as described by Aubourg et al. (1998). The relative fluorescence (RF) was
24 calculated as follows: RF = F/F_{st}, where F is the fluorescence measured at each
25 excitation/ emission maximum, and F_{st} is the fluorescence intensity of a quinine

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sulphate solution (1 µg ml^{-1} 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 (Bligh & Dyer, 1959).

Free fatty acid (FFA) content was determined in the lipid extract of the fish muscle by the Lowry and Tinsley (1976) method based on complex formation with cupric acetate-pyridine followed by spectrophotometric (715 nm) assessment. Results were expressed as g FFA kg^{-1} 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 (DOCE, 1989). 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 blue whiting.

Four categories were ranked (Table 1): 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 and flesh 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 obtained from the different microbial and chemical analyses were subjected to the ANOVA method ($p<0.05$) to explore differences by two different ways: processing

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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, microbial indices, lipid damage indices and sensory acceptance) was also carried out; for sensory values, the Spearman test was employed. Linear fittings are expressed; otherwise, the kind of fitting (quadratic or logarithmic) is mentioned.

3. RESULTS AND DISCUSSION

3.1. Microbial activity assessment

The comparative evolution of total aerobes for each batch during storage time can be seen in Figure 1. In general terms, the control batch exhibited higher microbial numbers than the counterpart batch stored in the natural-organic acid system. Thus, the most remarkable differences were observed on days 2, 5 and 9, the mean differences between batches being 0.57, 3.17 and 0.48 log CFU g^{-1} muscle, respectively. Nevertheless, in the course of the nine days of chilled storage, significant differences ($p<0.05$) for aerobe assessment were only observed at day 5. Concerning its evolution during the chilling storage, aerobe counts did not provide a good correlation value with chilling time.

The comparative evolution of psychrophilic bacteria can be seen in Figure 2. The counts in the fish batch processed under the natural-organic acid condition were lower than in the control batch from day 5 until day 9. Thus, the average differences in the counts of psychrophiles at day 5 were 1.44 log CFU g^{-1} muscle and for the following days differences of 0.46 and 0.91 log CFU g^{-1} muscle were determined. However, and although the natural-organic acid system exhibited a better control of bacterial growth, significant differences ($p<0.05$) were only obtained at day 5, as for aerobe assessment. Concerning its evolution during the chilling storage, psychrophilic counts did not

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provide a good correlation value with chilling time; compared to aerobe formation, good correlation was only obtained for the preserved batch ($r^2 = 0.94$), being bad in the case of control fish ($r^2 = 0.77$).

Enterobacteriaceae and proteolytic bacteria counts did not provide a clear tendency with chilling time (Table 2); thus, all values were included in the ranges 1.0-2.0 and 2.0-4.0 log CFU g^{-1} muscle, respectively. With respect to proteolytic bacteria, the natural-organic acid batch exhibited counts that were 0.37, 0.20 and 0.63 log CFU g^{-1} muscle lower than the control batch at days 2, 5 and 9, respectively. Likewise, the average counts for *Enterobacteriaceae* in the batch processed in the organic acid-mixture were 0.24, 0.35 and 0.30 log CFU g^{-1} muscle lower than the control batch at days 2, 5 and 9, respectively; however, no significant ($p>0.05$) differences were obtained between both batches for both microbiological assessments.

Lipolytic bacteria provided a constant value (2 log CFU g^{-1} muscle) for both batches during the 0-7-day period. At the end of the experiment, lipolytic counts were included in the 2.75-2.90 range, and no significant differences ($p>0.05$) were found between both batches.

The microbial preservative effects observed in this study were quite in accordance with the results reported by Kilinc et al. (2009), who studied the microbiology of rainbow trout (*Oncorhynchus mykiss*) fillets dipped in solutions including organic salts (acetate, lactate and citrate). Thus, these authors reported that the immersion of fillets in such solutions slowed down the proliferation of total aerobic and psycrophilic bacteria. Likewise, Marshall et al. (1996) investigated the microbiology of catfish (*Silurus glanis*) fillets treated by dipping in acetic acid and lactic acid solutions, and found a partial inhibition of bacterial growth under these conditions. An inhibitory effect of microbial activity was also found in chilled catfish (*Silurus glanis*) fillets that were

previously dipped in a lactic acid solution (Kim et al., 1995); as a result, an extension of the shelf life time was produced. Microbial activity was also partially inhibited by previous lactic acid treatment in chilled chub mackerel (*Scomber japonicus*) (Metin et al., 2001) and Mediterranean mackerel (*Scomber colias*) and mullet (*Mugil cephalus*) (Erkan, 2003) fillets according to a lower microbial count and total volatile-base nitrogen (TVB-N) content assessment throughout a 12-day and 9-day storage period, respectively.

In a previous experiment (Quitral et al., 2009), ice prepared from an aqueous solution from rosemary and oregano extracts was applied to a fatty fish species (Chilean jack mackerel; *Trachurus murphyi*). As in the present study, the employment of ice including a preservative compound as chilling medium led to a partial inhibition of microbial activity, according to the TVB-N content assessment.

In the present study, values obtained in all samples for all kinds of microbial parameters can be considered below the limit of acceptance. This conclusion agrees to previous research on microbial activity development during blue whiting chilling, where trimethylamine formation did not provide a great increase throughout a 8-day period (Smith et al., 1980; Rey-Mansilla et al., 1999) and a strong content of TVB-N content was only obtained after a 10-13-day chilled period (Aubourg et al., 2008).

3.2. Proximate analysis and pH assessment

Moisture and lipid contents were included in the ranges 797-831 and 3.8-4.4 (g kg^{-1} muscle), respectively. Such values agreed to previous results concerning this fish species (Dagbjartsson, 1975; Aubourg et al., 1998; Aubourg et al., 2007). No differences ($p>0.05$) in both constituents could be assessed as a result of the preservative treatment. However, a slight increasing moisture content ($p<0.05$) could be

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observed with chilling time for both preserved and control fish batches that could be explained as a result of contact with ice during the chilled storage.

Concerning the pH analysis, values were included in the range 6.90-7.00 for both kinds of fish samples throughout the whole experiment (Table 2). No significant differences ($p>0.05$) could be observed as a result of the preservative treatment, although lower mean values were observed in the 2-7-day period for the preserved batch, this in agreement to previously mentioned results on microbiological counts. Additionally, a slight increase in both batches could be observed in the 0-5-day period, so that a fair correlation value (r^2 = 0.86-0.89; logarithmic fitting) with chilling time could be depicted for the complete experiment.

Results obtained in the present study concerning pH assessment agree to previous research. Thus, a pH decrease was also obtained in chub mackerel (*Scomber japonicus*) (Metin et al., 2001) and catfish (*Silurus glanis*) (Kim et al., 1995) chilled fillets as a result of a previous lactic acid treatment. Additionally, pH value was studied in Chilean jack mackerel (*Trachurus murphyi*) that was kept under ice prepared from a water solution of rosemary and oregano extracts (Quitral et al., 2009); as a result, a lower pH increase could be observed when compared to control mackerel that was attributed to the presence in ice of preservative compounds from both plant extracts.

3.3. Lipid oxidation development

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

Primary oxidation provided very low scores, that remained in all cases under the 4.50 value (Table 3). The preservative treatment did not provide a clear effect ($p>0.05$) on

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peroxide formation; additionally, a clear tendency of peroxide formation could not be obtained as a result of the chilling time for both kinds of samples.

Concerning the secondary lipid oxidation development, again relatively low values were obtained, since lower scores than 0.60 were obtained in all cases (Table 3). When compared to control fish, the preservative treatment provided a lower ($p<0.05$) TBARS formation in chilled fish in the 7-9-day period. For both kinds of samples, a progressive increase ($p<0.05$) could be depicted during the chilled storage, so that good correlation values with chilling time ($r^2 = 0.89$-0.93; quadratic fitting) were obtained.

The fluorescent compound analysis provided a higher formation in control fish, this being significant ($p<0.05$) at days 2, 7 and 9 (Table 3); such results agree with the TBARS content, so that an inhibitory effect on lipid oxidation development was concluded for the preservative treatment. Starting fish showed a lower ($p<0.05$) FR value than the remaining samples for both batches; then (2-9-day period), a significant increase with chilling time increase was not observed ($p>0.05$) in any of the batches under study.

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 (Aubourg, 1993; Howell, 1995), or react with other molecules (nucleophilic-type, mostly) present in the fish muscle; consequently, the determination of each kind of compound cannot always provide an accurate method for the quality assessment. In the present research, no reliability for the primary lipid oxidation compound assessment (PV) was concluded, while a very short interaction compound formation (FR assessment) was produced. However, secondary (TBARS assessment) lipid oxidation compound measurement showed to be helpful in order to assess the lipid oxidation development throughout the whole chilled storage period.

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The partial inhibition of oxidation development found in the present research for chilled blue whiting as a result of the organic acid-mixture treatment in two steps agrees to previous research where AA and CA have shown a profitable antioxidant effect when applied as a preliminary treatment to further storage. Thus, CA and its salts have shown to play a synergist role with primary antioxidants and oxygen scavengers during the refrigerated (Stodolnik et al., 1992; Khalil & Mansour, 1998) and frozen (Pourashouri et al., 2009; Aubourg et al., 2004) storage of marine species. In the same way, AA and its salts have been reported to act as antioxidants in minced fish (Hwang & Regenstein, 1988), fish fillets (Badii & Howell, 2002) and whole fish (Aubourg et al., 2004) because of their oxygen scavenger and reducing roles.

Related to the second preservative step tested in the present research, ice prepared from a water solution from rosemary and oregano extracts was applied as chilling system to Chilean jack mackerel (*Trachurus murphyi*); as a result, a lower oxidation development (PV and TBARS assessments) in jack mackerel muscle could be observed during the chilled storage, being this inhibition attributed to polyphenolic compound presence in the icing system.

3.4. Lipid hydrolysis analysis

Lipid hydrolysis development was measured by the FFA content evolution (Figure 3). A lower FFA formation ($p<0.05$) could be concluded for the P-batch in the 7-9-day period when compared to control fish, so that an inhibitory effect of the preservative treatment could be concluded. For both batches, a marked FFA formation increase ($p<0.05$) could be outlined during chilling storage according to previous research (Aubourg et al., 1998; Quitral et al., 2009), so that a good correlation value with chilling time ($r^2 = 0.91$; logarithmic fitting) was obtained.

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FFA formation during chilling storage has been reported to be produced as a result of endogenous enzyme activity and microbial activity (Whittle et al., 1990; Madrid et al., 1994). Before the end of the microbial lag phase (up to 5-8 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 chilled blue whiting would show a first stage of FFA formation (0-5-day period) corresponding mostly to the endogenous enzymatic activity; in this period, no significant differences ($p>0.05$) between batches are found. Then (7-9-day period), when microbial activity should gain importance, fish corresponding to the P-batch remains at a relatively constant FFA level, while its counterpart C-batch fish shows a marked ($p<0.05$) FFA formation, so that a preservative effect would be accorded to the organic acid treatment.

To our knowledge, this inhibitory effect on FFA formation is described for the first time during the chilled storage of a lean fish species. As being molecules supporting acidulant properties, all three acids can be found responsible for this microbial inhibition (Whittle et al., 1990; Ashie et al., 1996). 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 (Kim et al., 1995; Metin et al., 2001; Gogus et al., 2006).

Additionally, FFA formation was studied in Chilean jack mackerel (*Trachurus murphyi*) that was kept under ice prepared from a water solution of rosemary and oregano extracts (Quitral et al., 2009); as a result, a lower FFA content could be observed when

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compared to control mackerel that was attributed to the presence of preservative compounds from both plants present in the icing system.

Relationship between lipid hydrolysis and oxidation has captivated a great attention. FFA are known to have detrimental effects on protein properties 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. In the present research, the best correlation value between FFA formation and the different lipid oxidation indices was obtained in the case of the TBARS assessment (r^2 = 0.83-0.87).

3.5. Sensory acceptance

A progressive acceptance decrease could be observed for both fish batches throughout the chilled storage, so that a good correlation value was obtained in all cases with chilling time (r^2 = 0.90-0.92; quadratic fitting). Preserved fish maintained a good quality till day 5 and were still acceptable at the end of the experiment; however, control fish was only considered as good quality till day 2 and showed a shelf life time of 7 days. Limiting attributes showed to be the external odour and gills appearance and odour.

Present research did not provide good correlation values for sensory acceptance and the different chemical lipid oxidation parameters, according to the fact that off-odours detected by the panel were putrid and not rancid. Thus, the best correlations were obtained with the TBA value (r^2 = 0.72-0.86). Previous research has shown a strong incidence of FFA accumulation on fish quality loss, being their presence associated in some extent to the lack of sensory acceptability and strongly interrelated with off-odour development (Ingemansson et al., 1995; Refsgaard et al., 2000). In this sense, present

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research provided a fair correlation value of sensory acceptance with FFA formation (r^2 = 0.81-0.90).

Present scores concerning the shelf life increase in chilled blue whiting as a result of the two-step organic acid treatment agree to previous research. Thus, CA and AA have shown to provide shelf life increases when employed as a pre-treatment (namely, aqueous solution dipping) to further frozen storage conditions (Pourashouri et al., 2009). Concerning the chilling storage, pre-treatment by dipping on a lactic acid solution also increased the shelf life in catfish (*Silurus glanis*) (Kim et al., 1995) and chub mackerel (*Scomber japonicus*) (Metin et al., 2001) fillets.

4. FINAL REMARKS

Assurance of both high quality and safety of chilled seafood is an important actual challenge for fish traders and food technologists, so that commercial requirements are always related to the search for valuable and practical technologies that may provide enhancing possibilities. The present study, focused to blue whiting chilling, provides a first approach to the employment of a novel two-step treatment including a natural organic acid-mixture (AA, CA and LA), such acids providing complementary preservative properties (acidulants, antioxidants and antimicrobians). In a first step, the acid mixture was applied in an aqueous dipping medium previously to the chilling storage; then, the acid mixture was included in the ice employed as chilling system.

According to quality indices related to microbiological activity (microbiological group counts) and lipid damage (hydrolysis and oxidation) development in chilled blue whiting, results obtained showed a profitable role of the organic acid-mixture treatment; thus, a partial inhibition of both damage pathways was attained, this leading to an increased shelf life time when compared to control fish.

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According to the results obtained in the present research, lipid hydrolysis showed to be a more relevant event than lipid oxidation in chilled blue whiting. Thus, FFA formation provided a great increase throughout the storage period, while lipid oxidation scores (peroxides and TBARS) remained relatively low. Meantime, counts obtained for the different microbiological groups remained as acceptable values throughout the whole experiment for individual fishes corresponding to both batches.

Further studies focused to the positive role of this two-step strategy are envisaged. A great effort ought to be addressed towards knowledge of the organic acid range where optimal effects could be attained, 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 both the dipping and the icing media to the fish muscle.

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LEGENDS TO FIGURES

Figure 1: Comparative aerobe count (log CFU g^{-1} muscle) assessment* in chilled blue whiting processed under different conditions**

* Mean values; standard deviations are denoted by bars.

** Processing conditions: C (control fish; fish dipped in water and then kept in traditional ice) and P (preserved fish; fish dipped in aqueous solution including 800 ppm of the organic acid-mixture and then kept in ice prepared from an aqueous 800 ppm solution of the acid mixture).

Figure 2: Comparative psychrotroph count (log CFU g^{-1} muscle) assessment* in chilled blue whiting processed under different conditions**

* Mean values are expressed; standard deviations are denoted by bars.

** Processing conditions as expressed in Figure 1.

Figure 3: Free fatty acid (g kg^{-1} lipids) assessment* in chilled blue whiting processed under different conditions**

* Mean values are expressed; standard deviations are denoted by bars.

** Processing conditions as expressed in Figure 1.

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TABLE 1

Scale employed for evaluating the sensory quality of chilled blue whiting

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
Flesh odour and taste	Sharply seaweedy; shellfish smell and taste	Weakly seaweedy; shellfish smell and taste	Incipiently putrid or ammonia odour and taste	Putrid or ammonia odour and taste

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1 **TABLE 2**

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4 **Evolution* of *Enterobacteriaceae* and proteolytic counts (log CFU g^{-1} muscle) and**

5 **pH values in chilled blue whiting processed under different conditions****

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Chilling Time (days)	*Enterobacteriaceae*		Proteolytics		pH	
	C	P	C	P	C	P
0	1.70 (0.01)		3.00 (0.06)		6.80 (0.12)	
2	1.24 (0.35)	1.00 (0.01)	3.22 (1.04)	2.85 (0.21)	6.92 (0.01)	6.85 (0.13)
5	1.50 (0.28)	1.15 (0.21)	2.50 (0.71)	2.30 (0.43)	7.00 (0.08)	6.97 (0.02)
7	0.99 (0.01)	1.73 (1.06)	1.99 (0.01)	2.80 (1.14)	6.98 (0.07)	6.96 (0.01)
9	1.93 (0.11)	1.63 (0.21)	3.58 (0.71)	2.95 (0.35)	6.96 (0.01)	6.96 (0.01)

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11 * Mean values; standard deviations are indicated in brackets. For each parameter and for
12 each chilling time, no significant (p>0.05) differences were obtained as a result
13 of processing.

14 ** Processing conditions: C (control fish; fish dipped in water and then kept in
15 traditional ice) and P (preserved fish; fish dipped in aqueous solution including
16 800 ppm of the organic acid-mixture and then kept in ice prepared from an
17 aqueous 800 ppm solution of the acid mixture).

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TABLE 3

Evolution of lipid oxidation* in chilled blue whiting processed under different conditions**

Chilling Time (days)	PV (meq active oxygen kg^{-1} lipids)		TBA value (mg malondialdehyde kg^{-1} muscle)		FR	
	C	P	C	P	C	P
0	1.53 (0.51)		0.06 (0.03)		0.73 (0.06)	
2	0.71 a (0.28)	1.58 b (0.43)	0.02 (0.01)	0.03 (0.01)	1.23 b (0.14)	0.83 a (0.02)
5	3.39 (1.11)	1.99 (1.18)	0.27 (0.15)	0.24 (0.03)	1.38 (0.26)	1.04 (0.26)
7	0.57 (0.44)	1.25 (0.46)	0.38 b (0.04)	0.21 a (0.05)	1.09 b (0.02)	0.86 a (0.15)
9	4.27 (0.44)	3.89 (1.38)	0.57 b (0.04)	0.39 a (0.11)	1.32 b (0.12)	0.97 a (0.17)

* Mean values; standard deviations are indicated in brackets. For each quality index and for each chilling time, mean values followed by different letters (a, b) denote significant differences as a result of processing. No letters are indicated when significant differences are not found ($p>0.05$).

** Processing conditions as expressed in Table 2.

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1 **TABLE 4**

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3 **Evolution of sensory quality* in chilled blue whiting processed under different**

4 **conditions****

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Processing condition	Chilling Storage Time (days)				
	0	**2**	**5**	**7**	**9**
C	E	A	B	B	C
P		A	A	B	B

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

11 ** Processing conditions as expressed in Table 2.

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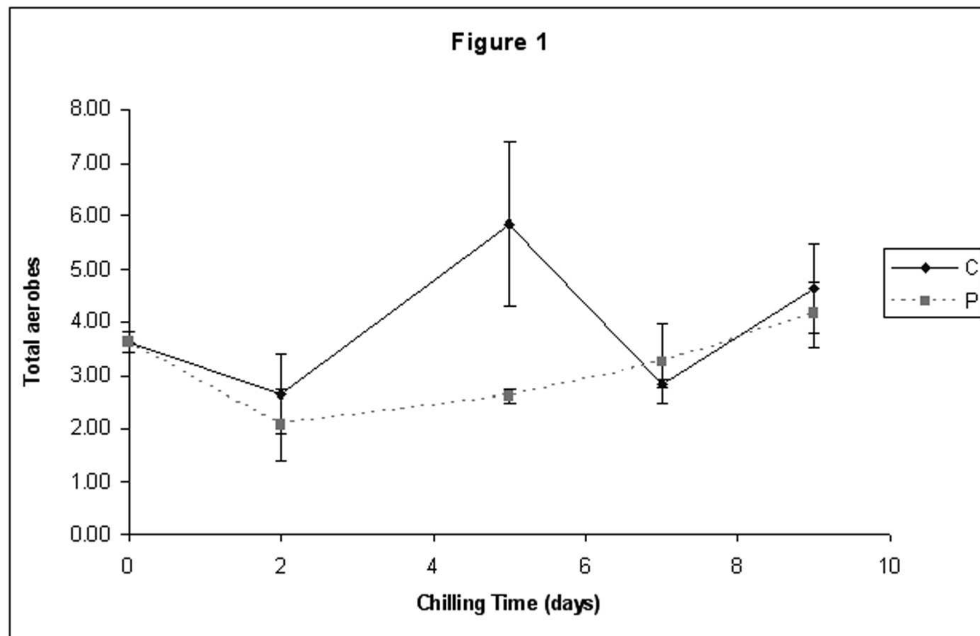

Figure 1
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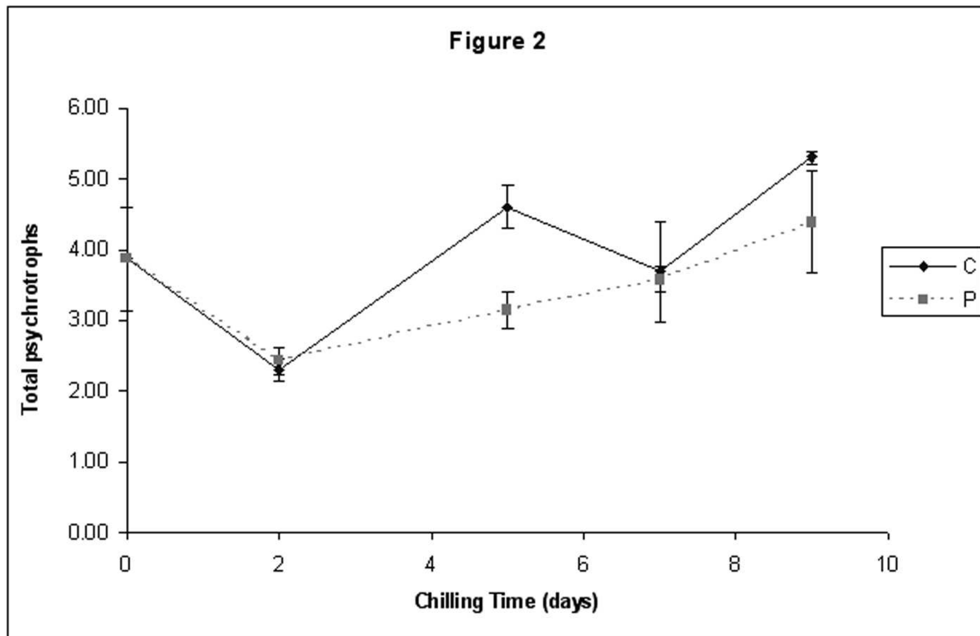

Figure 2
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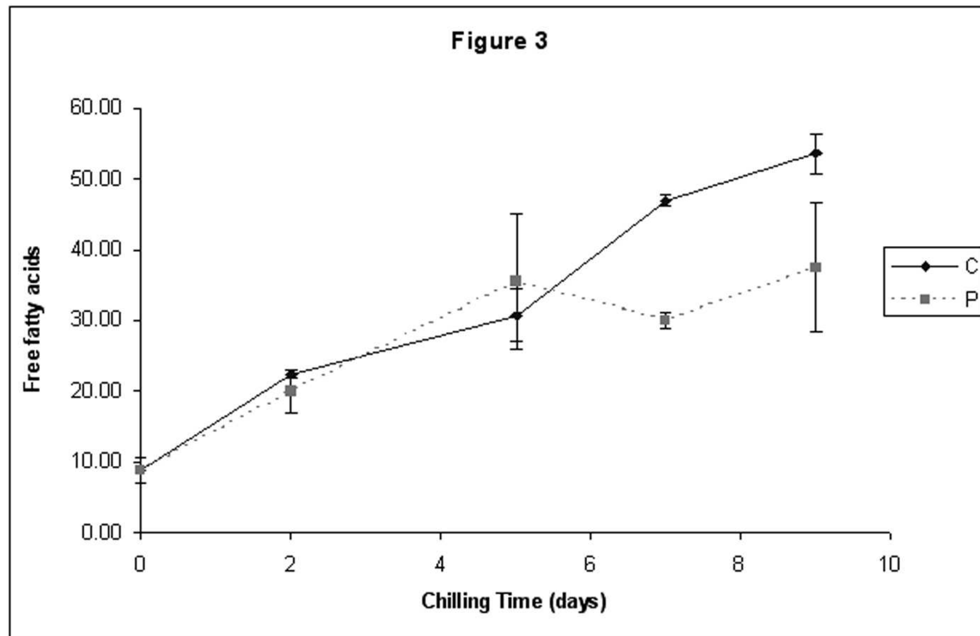

figure 3
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