Quality enhancement of chilled lean fish by previous active dipping in *Bifurcaria bifurcata* alga extract

José M. Miranda[1], Marcos Trigo[2], Jorge Barros-Velázquez[1], and Santiago P. Aubourg[2,*]

[1] Department of Analytical Chemistry, Nutrition and Food Science, School of Veterinary Sciences, University of Santiago de Compostela, Lugo (Spain).

[2] Department of Food Science and Technology, Marine Research Institute (CSIC), Vigo (Spain).

* Corresponding author. Phone: +34986231930; Fax: +34986292762; e-mail: saubourg@iim.csic.es

ABSTRACT

The present study addressed the quality enhancement of chilled fish by applying a preliminary dipping treatment containing a bioactive extract of the alga *Bifurcaria bifurcata*. Megrim (*Lepidorhombus whiffiagonis*) specimens were dipped in ethanolic-aqueous solutions containing two different concentrations of the alga extract (high: HAC batch and low: LAC batch). Two types of control batch were considered (ethanol-water dipping and no dipping). Microbial, chemical and sensory quality was monitored in fish throughout a 13-day storage period. An inhibitory effect ($p < 0.05$) of the dipping treatment (washing and the presence of alga extract) on microbial activity was observed, as determined by microbial (Enterobacteriaceae, lipolytic bacteria and psychrotrophs) and chemical (trimethylamine and free fatty acid formation) analysis. In most cases, this effect was found to be more intense for prolonged storage times and in fish specimens corresponding to the HAC batch. However, the combined effect of washing and the inclusion of alga extract in the dipping medium did not affect ($p > 0.05$) the rate of lipid oxidation events during storage. Interestingly, average scores revealed increased sensory quality in megrim corresponding to the HAC batch for prolonged storage (9–13 days). Consequently, the use of a bioactive extract of *B. bifurcata* alga as a preliminary dipping treatment can be considered of interest for further fish storage due to both its simplicity and its beneficial effects on fish quality enhancement.

Keywords: *Bifurcaria bifurcata*; dipping; chilled megrim; microbial activity; lipid damage; sensory acceptance

Running title: Chilled megrim quality and *B. bifurcata* dipping

INTRODUCTION

From the moment wild marine species are caught until they reach the final consumer, they are subject to a variety of handling and technological processes that can be decisive for the quality of the final product. To keep the original properties of the fish species and offer a high-quality fresh product, ice storage has been recognised as the most-employed method (Campos et al. 2012). However, because of the limited shelf-life of marine species, icing has been applied in combination with other preservative strategies such as ozone incorporation (Pastoriza et al. 2008), slurry-ice preparation (Yamada et al. 2002), salt addition (Huidobro et al. 1990), high-pressure treatment (Senturk and Alpas 2013) and the addition of natural compounds such as organic acids (Sallam 2007) or plant extracts (Özyurt et al. 2008).

In order to avoid the development of the different damage pathways (i.e., microbial activity, autolysis, lipid oxidation), a water dipping step has often been employed as a preliminary step prior to chilled storage, to remove blood, digestive juices, slime and faeces, and to partially prevent microbial contamination (López-Caballero et al. 2002; Erkan et al. 2007). To slow down the rate of such damage pathways and enhance preservation, in the last decade, certain preservative compounds such as sodium acetate (Manju et al. 2007) and ellagic acid alone or in combination with ascorbic acid (Zambuchini et al. 2008) have been incorporated in the dipping medium. Concerning refrigerated (2–4 ºC) fish, previous studies have considered the use of tea polyphenols and rosemary extract combined with chitosan (Li et al. 2012), carboxymethyl cellulose combined with *Zataria multiflora* essential oil and grape seed extract (Raeisi et al. 2015), alginate-based vitamin C solution combined with tea polyphenols (Song et al. 2011), fish gelatin and tea polyphenols (Feng et al. 2017) and

74 rosemary (*Rosmarinus officinalis*) combined with sage tea (*Salvia officinalis*) (Özogul
75 et al. 2011).

76 In the last decades, the use of seaweeds as food ingredients in Western countries
77 has received increasing attention due to their excellent nutritional and functional
78 properties (Rupérez 2002; Peinado et al. 2014). Seaweeds have been shown to be a
79 relevant source of beneficial constituents such as lipids, vitamins, trace minerals, dietary
80 fibre and amino acids (Díaz-Rubio et al. 2009; Paiva et al. 2014). Additionally,
81 seaweeds have attracted great attention because they contain a profitable variety of
82 chemical components with potential antimicrobial (Sandsdalen et al. 2003; Gupta &
83 Abu-Ghannam 2011) and antioxidant (Wang et al. 2010; Hallsdóttir et al. 2014)
84 activity. Consequently, seaweeds have been proposed as an alternative to the use of
85 synthetic compounds in food preservation strategies.

86 The objective of the present study was to improve fish quality during chilled
87 storage by the application of a preliminary dipping treatment containing a seaweed
88 extract. A brown alga (*Bifurcaria bifurcata*) was chosen on the basis of its great
89 availability in Western Europe and its relevant content of preservative compounds with
90 antioxidant and antimicrobial activity (Le Lann et al 2008; Zubia et al. 2009; Miranda et
91 al. 2016). In order to investigate such preservative effect on fish quality, megrim
92 (*Lepidorhombus whiffiagonis*), a fish species of relevant commercial value in Western
93 countries was chosen. Megrim specimens were dipped in ethanolic-aqueous solutions
94 containing different concentrations of an ethanolic-aqueous extract of *B. bifurcata*, and
95 then stored under chilled conditions.
96
97

MATERIALS AND METHODS

Extraction of lyophilised *B. bifurcata* and dipping media

The lyophilised alga *B. bifurcata* was provided by Porto-Muiños (Cerceda, A Coruña, Spain). A combined (i.e., ethanol and water) alga extract was applied as previously described (Barros-Velázquez et al. 2016); 66 g of lyophilised alga was mixed with absolute ethanol (2 × 528 mL), stirred for 30 s and centrifuged at 3,500 *xg* for 10 min at 4 °C. The supernatants were recovered, pooled together and diluted to 1 L with absolute ethanol. Then, the remaining lyophilised alga was mixed with distilled water (2 × 528 mL), stirred for 30 s and centrifuged at 3,500 *xg* for 10 min at 4 °C. The supernatants were recovered, pooled together and diluted to 1 L with distilled water.

Two different concentrations of the combined alga extract were considered, so that two kinds of dipping medium were prepared. For the more concentrated batch (HAC batch, more concentrated alga condition), 834 mL of the ethanol extract and 834 mL of the water extract were pooled together and adjusted to 11 L with distilled water (0.50% lyophilised alga/dipping solution, w/v). To prepare the less concentrated dipping medium (LAC batch, less concentrated alga condition), 166 mL of the ethanol extract and 166 mL of the water extract were pooled together and adjusted to make an 11-L solution with distilled water (0.10% lyophilised alga/dipping solution, w/v); previously, 668 mL of absolute ethanol was also included in this solution, so that the same amount of ethanol was present in both dipping media.

Two kinds of control batch were considered in this study. In the first, 834 mL of absolute ethanol was diluted to a 11-L solution with distilled water and employed as dipping control (DC batch) medium. In the other, a blank control batch with no dipping treatment (ND batch) was also taken into account.

Contents of lyophilised alga extracts in the dipping media employed in the current study were based on several preliminary tests carried out at our laboratory in the range of 0.02-2.00% lyophilised alga/dipping solution (w/v) (data not shown). An increasing presence of alga provided lower putrid odour and taste development. However, if a concentration higher than 0.50% was applied, undesirable modification of the external odour and colour of the whole fish occurred. Consequently, this concentration (0.50%) was considered for the HAC batch, together with a less concentrated batch (0.10%, LAC batch).

Fish sample collection, dipping and chilled storage

Fresh megrim (102 specimens) were obtained at Vigo harbour in north-western Spain and transported in ice to the laboratory. The length and weight of the individual fish were in the ranges 20 to 24 cm and 95 to 125 g, respectively.

Upon arrival at the laboratory, six specimens were separated and analysed to explore the initial quality at day 0. These specimens were divided into three groups (two specimens per group) which were analysed independently (n = 3). The remaining fish specimens were divided into four batches (24 specimens in each batch) which were subject to different dipping treatments. One batch (ND condition) was kept under chilled conditions without prior treatment. The remaining three batches were immersed either in DC, LAC or HAC dipping solutions, respectively, for 5 min in an isothermal room at 4 °C. After draining, all fish specimens were placed in ice and subject to chilled storage.

Boxes allowing drainage of melted ice were employed. In all cases, ice was renewed to maintain a 1:1 fish:ice ratio. All fish batches were stored for 13 days, samples being taken for analysis on days 2, 6, 9 and 13. At each sampling time, six

147 specimens were taken from each batch and divided into three groups (two specimens
148 per group) which were studied independently (n = 3).
149
150 **Analysis of lyophilised alga**
151 The total polyphenol content of lyophilised *B. bifurcata* was assessed by means of the
152 Folin–Ciocalteu colorimetric method (Cary 3E UV–Visible spectrophotometer, Varian,
153 Mulgrave, Victoria, Australia), as described previously (Rodríguez-Bernaldo de Quirós
154 et al. 2010). Measurements were made in triplicate. Gallic acid (GA) was used as
155 standard. Results were expressed as mg GA g^{-1} lyophilised alga.
156 The tocopherol compound content was determined in the lyophilised alga
157 according to the method of Cabrini et al. (1992). The presence of different tocopherol
158 compounds (α, β, γ and δ) was checked. Qualitative analysis was carried out by
159 employing the corresponding commercial standards. For quantitative analysis, content
160 was calculated with calibration curves prepared from the corresponding commercial
161 tocopherol. Results were expressed as mg kg^{-1} lyophilised alga.
162
163 **Microbiological evaluation of chilled megrim muscle**
164 Samples of 10 g of fish white muscle from chilled fish specimens were dissected under
165 aseptic conditions, mixed with 90 mL of 0.1% peptone water (Oxoid Ltd, London, UK),
166 and homogenised in a Stomacher blender (Seward Medical, London, UK), as previously
167 described (Ben-Gigirey et al. 1999). In all cases, serial dilutions of the microbial
168 extracts were prepared in 0.1% peptone water. Total aerobes were investigated by
169 surface inoculation in plate count agar (PCA; Oxoid) after incubation at 30 °C for 72 h.
170 Psychrotrophs were also investigated in PCA (Oxoid), incubation being carried out at
171 7–8 °C for 10 days. Enterobacteriaceae were assessed in crystal violet neutral red bile

glucose agar (VRBD agar; Merck, Darmstadt, Germany) after incubation at 37 °C for 24 h. Microorganisms exhibiting a proteolytic or lipolytic phenotype were determined on casein agar or tributyrin agar medium, respectively, after incubation at 30 °C for 48 h, as previously described (Ben-Gigirey et al. 2000).

In all cases, microbial counts were transformed into log colony-forming units (CFU) g^{-1} muscle before undergoing statistical analysis. All analyses were performed in triplicate.

Chemical evaluation of chilled megrim muscle

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

Trimethylamine-nitrogen (TMA-N) values were determined by the picrate method, as previously described (Tozawa et al. 1971). This technique involved the preparation of a 5% trichloroacetic acid extract of fish white muscle (10 g/25 mL). The results were expressed as mg TMA-N kg^{-1} flesh muscle.

Lipids were extracted from fish white muscle by the Bligh and Dyer (1959) method, based on single-phase solubilisation with a chloroform–methanol (1:1) mixture. Results were calculated as g lipid kg^{-1} muscle.

Free fatty acid (FFA) content in the lipid extract was determined by the Lowry and Tinsley (1976) method based on complex formation with cupric acetate-pyridine. Results were expressed as mg FFA kg^{-1} muscle.

Peroxide value (PV) in the lipid extract, expressed as meq active oxygen kg^{-1} lipids, was determined according to the ferric thiocyanate method (Chapman & McKay 1949).

196 Thiobarbituric acid index (TBA-i) was determined according to Vyncke (1970).
197 This method is based on the reaction between a trichloroacetic acid extract of fish white
198 muscle and thiobarbituric acid. The thiobarbituric acid reactive substances (TBARS)
199 content was spectrophotometrically measured at 532 nm, and calculated from a standard
200 curve using 1,1,3,3-tetraethoxy-propane (TEP). Results were expressed as mg
201 malondialdehyde kg^{-1} muscle.
202
203 **Sensory evaluation of chilled megrim**
204 The Quantitative Descriptive Analysis (QDA) method was carried out by a sensory
205 panel consisting of four to six experienced judges. Before sensory evaluation, the judges
206 received special training on chilled megrim, focusing on the evaluation of specimens
207 exhibiting different qualities. Special attention was paid to evolution of the sensory
208 descriptors that could act as limiting factors for shelf-life. Descriptors analysed were:
209 eyes, gills, external odour, skin appearance, texture, raw flesh odour, cooked flesh odour
210 and flesh taste. The different descriptors were evaluated on non-structural linear scales
211 with numerical scores from 7 to 0 (7.0, stage of highest quality; 0.0, stage of lowest
212 quality) in agreement with Aubourg et al. (2013). Four rank categories were considered
213 (European Council Regulation 1996): 7.0–6.1 (excellent), 6.0–4.0 (good), 3.9–1.1 (fair)
214 and 1.0–0.0 (rejectable).
215 At each sampling time, fish specimens from each batch were analysed.
216 Evaluation began by the analysis of raw fish, this being followed by analysis of cooked
217 samples. Cooking was accomplished at 95–100 °C for 7 min in a pre-warmed oven with
218 air circulation, and then samples were offered to the panel. At each sampling time,
219 whole fish specimens were coded with three-digit random numbers, presented to the
220 panellists in individual trays, and scored individually. Each descriptor for each sample

was scored a single session by each member of the panel. All descriptors were analysed at each sampling time, except for flesh taste, which was not studied on day 13. The panel members shared the samples tested. Results were expressed as average values of the different descriptors considered.

Statistical analysis

Data from the different quality measurements (microbial, chemical and sensory) were subject to one-way ANOVA ($p < 0.05$) to assess the effect of washing and the presence of alga extract in the dipping medium; comparison of means was performed using a least-square difference (LSD) method. Analyses were carried out using the PASW Statistics 18 software for Windows (SPSS Inc., Chicago, Il, USA).

RESULTS AND DISCUSSION

Microbiological results in chilled megrim muscle

Figure 1 shows the Enterobacteriaceae counts in all four batches during chilled storage. Thus, the numbers for this microbial group were in all cases below 4 log CFU g^{-1}, indicating remarkably high initial quality of the fish specimens. Significant ($p < 0.05$) differences were observed between ND and HAC batches on days 6 and 13, indicating an inhibitory effect on the growth of this microbial group derived from the washing process and the presence of the higher alga concentration in the dipping medium. Such differences reached a maximum of 1.14 log units after 6 days of chilled storage. Remarkably, no significant ($p > 0.05$) difference between the two control batches, ND and DC, was observed for any sampling time; however, average values were lower in the DC batch throughout the whole study.

Figure 2 illustrates the evolution of lipolytic bacteria in megrim muscle. This microbial group exerts a specific spoilage effect derived from the hydrolysis of lipids, mainly triglycerides and phospholipids, thus affecting fish quality. Interestingly, the assessment of lipolytic bacteria in megrim muscle during chilled storage revealed similar results to those for Enterobacteriaceae. Thus, the batch corresponding to dipping in the higher alga concentration exhibited significantly ($p < 0.05$) lower numbers of lipolytic bacteria compared with both of the control batches, ND and DC, on days 6 and 13. The greater differences between the HAC batch and the ND control batch rose up to 1.50 log units on day 6 of chilled storage. As in the case of Enterobacteriaceae, the inclusion of higher concentrations of alga extract in the dipping medium provided better protection of megrim quality than the batch including the lower alga concentration (i.e., average values), although the differences determined between the two alga batches, HAC and LAC, were not statistically significant ($p > 0.05$).

Table 1 shows the data corresponding to the growth of other microbial groups of relevance as regards fish quality and shelf-life in all four batches. With respect to aerobes, dipping in alga extracts did not provide a clear effect on microbial growth, and all differences observed between batches were non-significant ($p > 0.05$) except for those between LAC and ND batches on day 9, and between HAC and ND batches on day 13. Although slight protection derived from the washing process combined with the inclusion of alga extract could be inferred, such an effect was quite limited compared to that observed for other microbial groups.

With respect to psychrotrophs, the results were similar to those observed for aerobes. Thus, the HAC batch, corresponding to dipping in the higher concentration of alga extract, exhibited significantly ($p < 0.05$) lower psychrotroph counts than the ND

batch on days 6 and 9 of chilled storage (Table 1). Such differences reached a maximum of more than 1 log unit on day 6.

The evolution of bacteria able to produce extracellular proteases in megrim muscle during chilled storage is also displayed in Table 1. The growth of this microbial group, which includes specific spoilage microorganisms, negatively affects texture and other sensory aspects of fish quality during storage, thus limiting the shelf-life. In our study, dipping in a higher concentration of alga extract slowed down the growth of proteolytic bacteria. Thus, the HAC batch exhibited significantly ($p < 0.05$) lower concentrations of proteolytic bacteria than the ND batch on days 2 and 6, this revealing a protective effect of the washing process combined to the presence of the most concentrated alga extract for short storage periods. On contrary, longer storage periods (9-13 days) revealed equal or superior average values in batches including alga extracts as compared to both control batches.

In agreement with the above-mentioned results, an inhibitory effect on the development of microbial activity was produced as a result of previous dipping in solutions containing extract of the alga *B. bifurcata*. This result can be explained on the basis of two combined effects. On one hand, the washing effect can lead to a removal of blood, slime and other undesirable components present in the fish surface. On the other hand, this inhibitory effect can be justified by the presence of a high level of polyphenol compounds (40.8 ± 8.3 mg GA g^{-1} lyophilised alga) determined in this alga species, and the reported presence of different kinds of antimicrobial component, such as polyphenols, terpenes, hydroquinones, oligomeric phlorotannins and halogenated alkane and alkenes, in brown macroalgae (Smit 2004; Gupta & Abu-Ghannam 2011; Fleurence et al. 2012). Concerning *B. bifurcata*, the presence of bifuhalol (i.e., a polyhydroxyphenyl ether) was reported by Glombitza and Rössener (1974) on the basis

294 of NMR and IR spectroscopic analysis. Moreover, a recent study reported that the
295 inclusion of an ethanolic extract of this alga in the icing medium led to a reduction in
296 microbial numbers (aerobes, psychrotrophs, proteolytic and lipolytic bacteria and
297 Enterobacteriaceae) in chilled megrim muscle (Miranda et al. 2016).

298 Previous studies have shown an inhibitory effect of a previous water dipping
299 step on the development of microbial activity during the chilled storage of fish products.
300 Such preservative effect has been explained on the basis of the removal of microbial
301 flora in the fish surface, as well as slime, blood and other undesirable components, thus
302 providing a protecting coating to the fish surface. Thus, daily dipping of fish pieces in
303 tap water reduced the counts of microorganisms, namely total viable bacteria and H_2S-
304 producing microorganisms, in chilled gilthead seabream (*Sparus aurata*) stored for up
305 to 22 days (López-Caballero et al. 2002), as well as the levels of aerobes, psychrotrophs
306 and H_2S-producing bacteria in sea bream (*S. aurata*) stored for up to 13 days (Erkan
307 2007); in contrast, no effect on chemical parameters related to microbial activity (TVB-
308 N and TMA-N assessments) was reported in either study. Better results were obtained if
309 antimicrobial compounds were included in the dipping medium. In this case, in addition
310 to the above-mentioned advantages derived from water washing, antimicrobial
311 compounds would partially remain in the fish surface coating and provide an additional
312 protection against microbial development. Thus, Manju et al. (2007) reported
313 remarkable delays in fish spoilage, as determined by pH, TVB-N, TMA-N and total
314 viable counts, if sodium acetate was included in the dipping medium. Additionally,
315 dipping of fish in ellagic acid alone or in combination with ascorbic acid delayed the
316 proliferation of aerobes, psychrotrophs and pseudomonads in chilled sole (*Solea solea*)
317 stored for up to 8 days (Zambuchini et al. 2008).

318 A washing treatment with preservative effect was also described when slurry ice
319 was employed as the icing medium (Yamada et al. 2002; Losada et al. 2004). Thus, the
320 on-board application of slurry ice resulted in an inhibitory effect on microbial activity,
321 determined by lower aerobe, psychrotroph and proteolytic bacteria counts, in chilled
322 hake (*Merluccius merluccius*), angler (*Lophius piscatorius*) and ray (*Raja clavata*)
323 muscle (Barros-Velázquez et al. 2008).
324 Other studies have reported an inhibitory effect of a preliminary dipping step in
325 solutions containing an antimicrobial compound on microbial activity in packed fish
326 products stored under refrigeration (1-4 ºC). As in the case of chilled fish, a previous
327 dipping in a solution including antimicrobial compounds would provide the fish surface
328 with a coating susceptible to partially inhibit the microbial development during
329 refrigerated storage. Thus, a marked decrease was determined in total viable counts and
330 TVB-N values in refrigerated (20 days at 4 ºC) large yellow croaker (*Pseudosciaena*
331 *crocea*) if previously dipped in a solution including tea polyphenols and rosemary
332 extract combined with chitosan (Li et al. 2012). In another study, previous dipping in a
333 carboxymethyl cellulose incorporated with *Z. multiflora* essential oil and grape seed
334 extract led to an important inhibitory effect of total viable counts, lactic acid bacteria
335 and *Pseudomonas* spp. in refrigerated (20 days at 4 ºC) rainbow trout (*Oncorhynchus*
336 *mykiss*) (Raeisi et al. 2015). Inhibition of biogenic amines (i.e., histamine, putrescine
337 and cadaverine), ammonia and TMA formation resulted from previous dipping in
338 rosemary (*R. officinalis*) and sage tea (*S. officinalis*) extracts when applied to
339 refrigerated (3 ± 1 °C for 20 days) vacuum-packaged sardine (*Sardina pilchardus*)
340 fillets (Özogul et al. 2011). Likewise, a previous dipping step in a solution containing
341 sodium acetate, lactate and citrate was also beneficial for inhibiting the proliferation of
342 aerobics, psychrotrophs, *Pseudomonas* spp., H_2S-producing bacteria, lactic acid bacteria

and Enterobacteriaceae in refrigerated (15 days at 1 ºC) salmon (*O. nerka*) slices (Sallam 2007). A chitosan/gelatine coating proved to prevent the development of total viable counts, yeasts, moulds as well as the formation of TVB-N compounds in golden pomfret (*Trachinotus blochii*) fillets during a 17-day refrigeration period at 4 ºC (Feng et al. 2016). Furthermore, an edible coating including fish gelatine and tea polyphenols led to a marked antimicrobial effect (inhibition of aerobes, psychrotrophs, yeasts and moulds, and lower increases of pH and TMA values) in refrigerated (17 days at 4 ± 1 ºC) tilapia (*Oreochromis niloticus*) fillets (Feng et al. 2017). Finally, lower counts of total viable, Enterobacteriaceae, lactic acid bacteria and H_2S-producing bacteria as well as an inhibitory effect on pH and TVB-N formation were observed in refrigerated (4 °C for 15 days) rainbow trout (*Oncorhynchus mykiss*) previously dipped in carrageenan, alone or in combination with essential lemon oil (Volpe et al. 2015).

Chemical results in chilled megrim muscle

Microbial activity was also measured in an indirect way by means of two chemical indices (i.e., pH and TMA-N content). A general pH increase was observed in all batches as storage time progressed (Table 2). Comparison of the different fish batches showed only slight significant differences ($p < 0.05$). However, fish corresponding to the ND batch revealed a higher average pH value throughout the whole study.

A marked increase in TMA-N content could be observed in all batches throughout chilled storage (Figure 3), significant differences ($p < 0.05$) between batches being found as a result of dipping in the alga extracts. Thus, fish corresponding to the LAC batch exhibited lower TMA-N values than both controls after 2 and 9 days of storage. Moreover, the HAC batch also exhibited slower TMA-N formation than the

counterpart controls in the 9–13-day period. Interestingly, no significant differences ($p > 0.05$) between fish corresponding to the two control batches were observed.

Increases in the pH value of fish muscle indicate the accumulation of alkaline compounds, such as ammonia, TMA and other nitrogenous compounds, which are mainly derived from microbial activity (Sandsdalen et al., 2003; Campos et al., 2012). Furthermore, during the storage of fish under refrigeration conditions, TMA is produced as a result of trimethylamine oxide (TMAO) breakdown by microbial TMAO reductase. Previous research concerning formation of such kind of compounds has been mentioned above when describing previous reports related to the effect of previous dipping with/without antimicrobial compounds on microbial development.

FFA formation also progressed with storage time in all batches (Table 2). Comparison between batches revealed lower average values in fish corresponding to the HAC batch for the 6-13-day period. Interestingly, differences were significant ($p < 0.05$) at the end of storage when compared to both control batches. Both endogenous enzyme presence and microbial activity have been signalled as responsible for FFA formation during the chilled storage of fish (Campos et al. 2012). Before the end of the microbial lag phase (about 6–9 days), endogenous enzyme activity should be predominant; after that time, microbial activity should gain importance and be mostly responsible for the development of lipid hydrolysis. The present results show an inhibitory effect on FFA formation at the end of the experiment in fish corresponding to the HAC batch, so that inhibition of microbial activity at that time is implied as a result of the presence of alga extract in the dipping medium.

This inhibitory effect of *B. bifurcata* was already proved by Miranda et al. (2016); thus, the inclusion of an ethanolic-aqueous extract in the icing medium, led to lower levels of FFA in chilled megrim (*L. whiffiagonis*). Furthermore, lipid hydrolysis

development was also inhibited if an ethanolic-aqueous extract of *Cystoseira compressa* was included in the icing medium employed during horse mackerel (*Trachurus tracurus*) chilling storage (Oucif et al. 2018). Finally, FFA formation was slowed down in chilled Chilean jack mackerel (*Trachurus murphyi*) by including an aqueous extract of oregano or rosemary in the icing system (Quitral et al 2009).

Lipid oxidation was estimated by means of peroxide and TBA indices (Table 2). Interestingly, little development of lipid oxidation was concluded in all batches during storage. Concerning peroxides, values were in all cases below 2.6. Remarkably, lower average PV were obtained in fish corresponding to the blank control (ND batch) throughout the whole study. For the TBA-i, slight differences ($p > 0.05$) between batches were observed. Consequently, an inhibitory effect on lipid oxidation could not be implied as a result of previous dipping in alga solutions.

Tocopherol and polyphenol compounds in general have been described to be able to inhibit lipid oxidation by the stabilisation of free radicals responsible for the advance of such damage pathway. On the basis of the presence of tocopherol compounds (475.1 ± 0.3, 152.6 ± 0.9 and 122.5 ± 2.7 mg kg^{-1} lyophilised alga for alpha, gamma and delta tocopherol, respectively) and of the content of the above-mentioned polyphenol compounds (40.8 ± 8.3 mg GA g^{-1} lyophilised alga) obtained for the current lyophilised alga, an inhibitory effect on this damage pathway was expected to occur in the current lean fish species (lipid content of white muscle: 4.0–4.7 g kg^{-1}).

Contrary to the present research, previous studies have reported antioxidant properties and activity of *B. bifurcata* extracts. Thus, different polyhydroxyphenyls and phenylethers were isolated and their structures established by NMR and IR spectroscopy by Glombitza et al. (1976). On the basis of different *in vitro* assays (DPPH, reducing activity and beta-carotene methods), the antioxidant activity of

417 different kinds of *B. bifurcata* extracts has been reported (Connan et al. 2007; Le Lann
418 et al. 2008; Zubia et al. 2009). Interestingly, the inclusion of an ethanolic extract of *B.*
419 *bifurcata* in the icing medium has been shown to produce an inhibitory effect on the
420 development of lipid hydrolysis (FFA formation) and oxidation (TBA value and
421 fluorescent compounds formation) in chilled megrim (Miranda et al. 2016). Recently,
422 the presence of an aqueous *B. bifurcata* extract in the dipping medium of canned
423 mackerel (*Scomber scombrus*) led to inhibition of the development of lipid oxidation
424 (fluorescent compounds formation) in fish muscle (Barbosa et al. 2018); such an effect
425 increased in parallel with the concentration of alga extract present in the canned
426 product.

427 A preliminary dipping step with a sodium acetate solution has been reported to
428 lead to less oxidation (TBA value assessment) in vacuum-packaged pearlspot (*Etroplus*
429 *suratensis*) during chilled (up to 15 days) storage (Manju et al. 2007). Likewise, the
430 washing effect derived from the application of slurry ice also led to an inhibitory effect
431 on lipid hydrolysis (FFA formation) and oxidation (PV and fluorescent compound
432 formation) (Losada et al. 2004).

433 Interestingly, inhibition of lipid oxidation was also observed in refrigerated fish
434 previously submitted to preliminary dipping steps in solutions containing antioxidant
435 compounds. Thus, lipid oxidation was slowed down in chilled large yellow croaker *(P.*
436 *crocea)* dipped in a solution containing tea polyphenols and rosemary extract combined
437 with chitosan (PV and TBA value assessments; Li et al. 2012). Similar results were
438 observed in refrigerated (4 °C up to 20 days) rainbow trout *(O. mykiss)* previously
439 coated with carboxymethyl cellulose containing *Z. multiflora* essential oil and grape
440 seed extract (TBA value assessment; Raeisi et al. 2015). Likewise, lipid damage was
441 partially inhibited in refrigerated bream (*Megalobrama amblycephala*) previously

dipped in an alginate-based solution containing vitamin C and tea polyphenols (TBA value assessment) (Song et al. 2011). Finally, lipid damage events were slowed down in chilled salmon (*O. nerka*) slices preliminarily dipped in sodium acetate and citrate solutions (PV and TBA value assessments) (Sallam 2007).

Sensory acceptance of chilled megrim

Progressive sensory quality loss was observed in all batches as storage time increased (Figure 4). Thus, all batches were scored as "good quality" for the 2-6-day period, except for fish specimens corresponding to the ND batch, which were considered of "fair quality" on day 6. Although scarce significant differences ($p < 0.05$) were observed, comparison among samples reflected higher average scores in most cases in fish specimens dipped in the most concentrated alga extract (at days 2, 9 and 13). Differences were found to be significant ($p < 0.05$) at the end of the storage time so that a sensory quality enhancement was concluded at that time as a result of the washing process and the presence of the higher alga concentration. In agreement with microbial and chemical parameters, the sensory quality enhancement can be explained by the inhibitory effect on microbial activity. Thus, fish specimens belonging to the HAC batch were still acceptable after 13 days of storage (score 1.3), while all other batches were considered as not acceptable (scores below 1.0). Remarkably, higher average scores were given to fish specimens belonging to the DC batch when compared to their counterparts from the ND batch. However, differences were not found to be significant ($p > 0.05$). Among the different individual descriptors analysed, eyes, gills and external odour were found to be the limiting factors.

No effect of daily dipping in tap water on the sensory quality of chilled sea bream (*S. aurata*) pieces has been reported (Erkan 2007). However, other studies

467 indicate that when preservative compounds are included in the dipping medium, sensory
468 acceptance increases. Thus, an increase in shelf-life parameters (appearance, texture,
469 odour and flavour) of pearlspot (*E. suratensis*) was observed when comparing air-
470 packed samples (8 days) with vacuum-packed samples (10 days) and with acetate-
471 treated vacuum-packaged samples (15 days) (Manju et al. 2007). Furthermore, an
472 increase in shelf-life was also observed in chilled sole (*S. solea*) previously dipped in
473 ellagic acid alone or in combination with ascorbic acid (10 days) when compared with a
474 control fish batch (8 days) (Zambuchini et al. 2008).

475 A positive effect of washing on sensory acceptance was also reported to be
476 derived from the application of slurry ice to different fish species. In this sense, an
477 increase in shelf-life was observed during the on-board chilled storage of hake (*M.*
478 *merluccius*) (limiting descriptor: consistency), angler (*L. piscatorius*) (limiting
479 descriptor: ventral cavity) and ray (*R. clavata*) (limiting descriptor: external ammonia
480 odour) (Barros-Velázquez et al. 2008), as well as during the in-land chilled storage of a
481 fatty fish species such as sardine (Losada et al. 2004).

482 An increase in shelf-life has also been reported for refrigerated (1–4 °C) fish
483 previously subject to different kinds of dipping media. Thus, previous reports account
484 for refrigerated large yellow croaker (*P. crocea*) previously coated with tea polyphenols
485 and rosemary extract combined with chitosan (quality index method, QIM; Li et al.
486 2012), refrigerated rainbow trout (*O. mykiss*) previously coated with carrageenan alone
487 or in combination with essential lemon oil (visual inspection of general aspect; Volpe et
488 al. 2015), and refrigerated salmon (*O. nerka*) slices previously dipped in a sodium
489 acetate, lactate and citrate solution (Sallam 2007).

490

491

CONCLUSIONS

Enhancement of the quality of chilled megrim was achieved by means of a combined treatment including washing in an ethanolic-aqueous solution and the inclusion of a bioactive *B. bifurcata* extract. Such treatment provided an inhibitory effect ($p < 0.05$) on spoilage mechanisms, as determined by microbial (Enterobacteriaceae, lipolytic and psychrotrophic bacteria) and chemical (TMA and FFA formation) analysis. This effect was found in specimens corresponding to the most concentrated alga extract (HAC batch) and was more intense, in most cases, at advanced storage times. However, the presence of the alga extract in the dipping medium did not affect ($p > 0.05$) the rate of lipid oxidation events during storage. Interestingly, average scores revealed better maintenance of sensory quality in megrim corresponding to the HAC batch for the 9-13-day period. Remarkably, a significant effect was found at the end of the experiment as a result of both washing and inclusion of alga extract in the dipping medium. As a result of the inhibitory effect on the microbial activity, the dipping treatment proposed in this study may have practical application for both on-board and in-land fish storage strategies due to its beneficial effects on fish quality and the simple methodology employed. Further research would be necessary to optimise this preservative role when applied to different kinds of seafood.

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REFERENCES

Aubourg, S. P., Rodríguez, A., Sierra, Y., Tabilo-Munizaga, G., & Pérez-Won, M. (2013). Sensory and physical changes in chilled farmed Coho salmon (*Oncorhynchus kisutch*): Effect of previous optimised hydrostatic high-pressure conditions. *Food and Bioprocess Technology*, *6*, 1539-1549.

Barbosa, R. G., Trigo, M., Fett, R., & Aubourg, S. P. (2018). Impact of a packing medium with alga *Bifurcaria bifurcata* extract on canned Atlantic mackerel (*Scomber scombrus*) quality. *Journal of the Science of Food and Agriculture*. DOI: 10.1002/jsfa.8861.

Barros-Velázquez, J., Gallardo, J. M., Calo, P., & Aubourg, S. P. (2008). Enhanced quality and safety during on-board chilled storage of fish species captured in the Grand Sole North Atlantic fishing bank. *Food Chemistry*, *106*, 493-500.

Barros-Velázquez, J., Miranda, J. M., Ezquerra-Brauer, J. M., & Aubourg, S. P. (2016). Impact of icing systems with aqueous, ethanolic and ethanolic-aqueous extracts of alga *Fucus spiralis* on microbial and biochemical quality of chilled hake (*Merlucius merluccius*). *International Journal of Food Science and Technology*, *51*, 2081-2089.

Ben-Gigirey, B., Vieites Baptista de Sousa, J., Villa, T., & Barros-Velázquez, J. (1999). Histamine and cadaverine production by bacteria isolated from fresh and frozen albacore (*Thunnus alalunga*). *Journal of Food Protection*, *62*, 933–939.

Ben-Gigirey, B., Vieites Baptista de Sousa, J., Villa, T., & Barros-Velázquez, J. (2000). Characterization of biogenic amine-producing *Stenotrophomonas maltophilia* strains isolated from white muscle of fresh and frozen albacore tuna. *International Journal of Food Microbiology*, *57*, 19-31.

542 Bligh, E., & Dyer, W. (1959). A rapid method of total extraction and purification.
543 *Canadian Journal of Biochemistry and Physiology*, *37*, 911-917.
544 Cabrini, L., Landi, L., Stefanelli, C., Barzanti, V., & Sechi, A. (1992). Extraction of
545 lipid and lipophilic antioxidants from fish tissues: A comparison among different
546 methods. *Comparative Biochemistry and Physiology. Biochemistry and*
547 *Molecular Biology, 101*, 383-386.
548 Campos, C., Gliemmo, M., Aubourg, S. P., & Barros-Velázquez, J. (2012). Novel
549 technologies for the preservation of chilled aquatic food products. In A.
550 McElhatton, & P. Amaral Sobral (Eds.), *Novel Technologies in Food Science*
551 (pp. 299-323), chapter 13. New York, USA: Springer.
552 Chapman, R., & McKay, J. (1949). The estimation of peroxides in fats and oils by the
553 ferric thiocyanate method. *Journal of the American Oil Chemists' Society*, *26*,
554 360-363.
555 Connan, S., Deslandes, E., & Gall, E. (2007). Influence of day-night and tidal cycles on
556 phenol content and antioxidant capacity in three temperate intertidal brown
557 seaweeds. *Journal of Experimental Marine Biology and Ecology, 349*, 359-369.
558 Díaz-Rubio, MªE., Pérez-Jiménez, J., & Saura-Calixto, F. (2009). Dietary fiber and
559 antioxidant capacity in *Fucus vesiculosus* products. *International Journal of*
560 *Food Science and Nutrition, 60*, 23-34.
561 Erkan, N. (2007). Sensory, chemical, and microbiological attributes of sea bream
562 (*Sparus aurata*): Effect of washing and ice storage. *International Journal of*
563 *Food Properties, 10*, 421-434.
564 European Council Regulation (1996). European Community (EC), No 2406/96, 26
565 November 1996. *Official Journal of European Communities*, L-334/2:23.12.

566 Feng, X., Bansal, N., & Yang, H. (2016). Fish gelatin combined with chitosan coating
567 inhibits myofibril degradation of golden pomfret (*Trachinotus blochii*) fillet
568 during cold storage. *Food Chemistry*, *200*, 283-292.
569 Feng, X., Ng, V. K., Mikš-Krajnik, M., & Yang, H. (2017). Effects of fish gelatin and
570 tea polyphenol coating on the spoilage and degradation of myofibril in fish fillet
571 during cold Storage. *Food and Bioprocess Technology*, *10*, 89-102.
572 Fleurence, J., Morançais, M., Dumay, J., Decottignies, P., Turpin, V., Munier, M.,
573 García-Bueno, N., & Jaouen, P. (2012). What are the prospects for using
574 seaweed in human nutrition and for marine animals raised through aquaculture?
575 *Trends in Food Science and Technology*, *27*, 57-61
576 Glombitza, K., & Rösener, H. (1974). Bifuhalol: Ein diphenyläter aus *Bifurcaria*
577 *bifurcata*. *Phytochemistry*, *13*, 1245-1247.
578 Glombitza, K., Rösener, H., & Koch, M. (1976). Polyhydroxyoligophenyle und
579 phenyläther aus *Bifurcaria bifurcata*. *Phytochemistry*, *15*, 1279-1281.
580 Gupta, S., & Abu-Ghannam, N. (2011). Bioactive potential and possible health effects
581 of edible brown seaweeds. *Trends in Food Science and Technology*, *22*, 315-
582 326.
583 Halldorsdóttir, S., Sveinsdóttir, H., Gudmundsdóttir, A., Thorkelsson, G., &
584 Kristinsson, H. (2014). High quality fish protein hydrolysates prepared from by-
585 product material with *Fucus vesiculosus* extract. *Journal of Functional Foods*, *9*,
586 10-17.
587 Huidobro, A., Montero, P., Tejada, M., Colmenero, F., & Borderías, J. (1990). Changes
588 in protein function of sardines stored in ice with and without added salt.
589 *Zeitschrift für Lebensmittel Untersuchung und Forschung*, *190*, 195-198.

590 Le Lann, K., Jégou, C., & Stiger-Pouvreau, V. (2008). Effect of different conditioning
591 treatments on total phenolic content and antioxidant activities in two
592 Sargassacean species: Comparison of the frondose *Sargassum muticum* (Yendo)
593 Fensholt and the cylindrical *Bifurcaria bifurcata* R. Ross. *Phycological*
594 *Research, 56*, 238-245.
595 Li, T., Hub, W., Li, J., Zhang, X., Zhu, J., & Li, X. (2012). Coating effects of tea
596 polyphenol and rosemary extract combined with chitosan on the storage quality
597 of large yellow croaker (*Pseudosciaena crocea*). *LWT-Food Science and*
598 *Technology, 25*, 101-106.
599 López-Caballero, MªE., Huidobro, A., Pastor, A., & Tejada, M. (2002). Microflora of
600 gilthead seabream (*Sparus aurata*) stored in ice. Effect of washing. *European*
601 *Food Research and Technology, 215*, 393-400.
602 Losada, V., Piñeiro, C., Barros-Velázquez, J., & Aubourg, S. P. (2004). Effect of slurry
603 ice on chemical changes related to quality loss during European hake
604 (*Merluccius merluccius*) chilled storage. *European Food Research and*
605 *Technol*ogy, *219*, 27-31.
606 Lowry, R., & Tinsley, I. (1976). Rapid colorimetric determination of free fatty acids.
607 *Journal of the American Oil Chemists' Society*, *53*, 470-472.
608 Manju, S., Leema Jose, Srinivasa Gopal, T. K., Ravishankar, C.N., & Lalitha, K.
609 (2007). Effects of sodium acetate dip treatment and vacuum-packaging on
610 chemical, microbiological, textural and sensory changes of Pearlspot (*Etroplus*
611 *suratensis*) during chill storage. *Food Chemistry, 102*, 27-35.
612 Miranda, J. M., Ortiz, J., Barros-Velázquez, J., & Aubourg, S. P. (2016). Quality
613 enhancement of chilled fish by including alga *Bifurcaria bifurcata* extract in the
614 icing medium. *Food and Bioprocess Technology, 9*, 387-395.

615 Oucif, H., Miranda, J. M., Ali Mehidi, S., Abi-Ayad, S. M., Barros-Velázquez, J., &
616 Aubourg, S. 2018. Effectiveness of a combined ethanol-aqueous extract of alga
617 *Cystoseira compressa* for the quality enhancement of a chilled fatty fish species.
618 *European Food Research and Technology, 244*, 291-299.
619 Özogul, F., Kuley, E., & Kenar, M. (2011). Effects of rosemary and sage tea extracts on
620 biogenic amines formation of sardine (*Sardina pilchardus*) fillets. *International*
621 *Journal of Food Science and Technology, 46*, 761-766.
622 Özyurt, G., Kuley, E., Balikçi, E., Kaçar, Ç., Gökdogan, S., Etyemez, M., & Özogul, F.
623 (2012). Effect of the icing with rosemary extract on the oxidative stability and
624 biogenic amine formation in sardine (*Sardinella aurita*) during chilled storage.
625 *Food and Bioprocess Technology, 5*, 2777-2786.
626 Paiva, L., Lima, E., Ferreira Patarra, R., Neto, A., & Baptista, J. (2014). Edible Azorean
627 macroalgae as source of rich nutrients with impact on human health. *Food*
628 *Chemistry, 164*, 128-135.
629 Pastoriza, L., Bernárdez, M., Sampedro, G., Cabo, M., & Herrera, J. (2008). The use of
630 water and ice with bactericide to prevent onboard and onshore spoilage of
631 refrigerated megrim (*Lepidorhombus whiffiagonis*). *Food Chemistry, 110*, 31-38.
632 Peinado, I., Girón, J., Koutsidis, G., & Ames J. M. (2014). Chemical composition,
633 antioxidant activity and sensory evaluation of five different species of brown
634 edible seaweeds. *Food Research International, 66*, 36-44.
635 Raeisi, M., Tajik, H., Aliakbarlu, J., Hamed Mirhosseini, S., & Mohammad Hashem
636 Hosseini, S. (2015). Effect of carboxymethyl cellulose-based coatings
637 incorporated with *Zataria multiflora* Boiss. essential oil and grape seed extract
638 on the shelf life of rainbow trout fillets. *LWT-Food Science and Technology, 64*,
639 898-904.

Rodríguez-Bernaldo de Quirós, A., Frecha-Ferreiro, S., Vidal-Pérez, A., & López-Hernández, J. (2010). Antioxidant compounds in edible brown seaweeds. *European Food Research and Technology, 231*, 495-498.

Rupérez, P. (2002). Mineral content of edible marine seaweeds. *Food Chemistry, 79*, 23-26.

Sallam, K. (2007). Antimicrobial and antioxidant effects of sodium acetate, sodium lactate, and sodium citrate in refrigerated sliced salmon. *Food Control, 18*, 566-575.

Sandsdalen, E., Haug, T., Stensvag, K., & Styrvold, O. (2003). The antibacterial effect of a polyhydroxylated fucophlorethol from the marine brown alga, *Fucus vesiculosus*. *World Journal of Microbiology and Biotechnology, 19*, 777-782.

Senturk, T., & Alpas, H. (2013). Effect of high hydrostatic pressure treatment (HHPT) on quality and shelf life of Atlantic mackerel (*Scomber scombrus*). *Food and Bioprocess Technology, 6*, 2306-2318.

Smit, A. (2004). Medicinal and pharmaceutical uses of seaweed natural products: A Review. *Journal of Applied Phycology, 16*, 245-262.

Song, Y., Liu, L., Shen, H., You, J., & Luo, Y. (2011). Effect of sodium alginate-based edible coating containing different anti-oxidants on quality and shelf life of refrigerated bream (*Megalobrama amblycephala*). *Food Control, 22*, 608-615.

Tozawa, H., Erokibara, K., & Amano, K. (1971). Proposed modification of Dyer's method for trimethylamine determination in codfish. In R. Kreuzer (Ed.), *Fish Inspection and Quality Control* (pp. 187-190). London, UK: Fishing News Books Ltd.

Volpe, M., Siano, F., Paolucci, M., Sacco, A., Sorrentino, A., Malinconico, M., & Varricchio, E. (2015). Active edible coating effectiveness in shelf-life

665 enhancement of trout (*Oncorhynchus mykiss*) fillets. *LWT-Food Science and*
666 *Technology, 60*, 615-622.
667 Vyncke, W. (1970). Direct determination of the thiobarbituric acid value in
668 trichloracetic acid extracts of fish as a measure of oxidative rancidity. *Fette*
669 *Seifen Anstrichmittel, 72*, 1084-1087.
670 Wang, T., Jónsdóttir, R., Kristinsson, H., Thorkelsson, G., Jacobsen, C., Yuca
671 Hamaguchi, P., & Olafsdóttir, G. (2010). Inhibition of haemoglobin-mediated
672 lipid oxidation in washed cod muscle and cod protein isolates by *Fucus*
673 *vesiculosus* extract and fractions. *Food Chemistry, 123*, 321-330.
674 Yamada, M., Fukusako, S., & Kawanami, T. (2002). Performance analysis on the
675 liquid-ice thermal storage system for optimum operation. *International Journal*
676 *of Refrigeration, 25*, 267-277.
677 Zambuchini, B., Fiorini, D., Verdenelli, M.C., Orpianesi, C., & Ballini, R. (2008).
678 Inhibition of microbiological activity during sole (*Solea solea* L.) chilled storage
679 by applying ellagic and ascorbic acids. *LWT-Food Science and Technology, 41*,
680 1733-1738.
681 Zubia, M., Fabre, M. S., Kerjean, V., Le Lann, K., Stiger-Pouvreau, V., Fauchon, M., &
682 Deslandes, E. (2009). Antioxidant and antitumoral activities of some Phaeophyta
683 from Brittany coasts. *Food Chemistry, 116*, 693-701.
684

FIGURE LEGENDS

Figure 1: Effect of various dipping conditions* on Enterobacteriaceae counts (log CFU g^{-1} muscle) in megrim muscle throughout subsequent chilled storage**

* Abbreviations of dipping conditions as expressed in Table 1.

** Average values of three (n = 3) replicates; standard deviations are indicated by bars. Average values accompanied by different letters (a, b) denote significant differences ($p < 0.05$) as a result of the dipping medium applied. No letters are included when significant differences were not found ($p > 0.05$). Initial fish value: 1.00 ± 0.00 log CFU g^{-1} muscle.

Figure 2: Effect of various dipping conditions* on lipolytic bacteria counts (log CFU g^{-1} muscle) in megrim muscle throughout subsequent chilled storage**

* Abbreviations of dipping conditions as expressed in Table 1.

** Average values of three (n = 3) replicates; standard deviations are indicated by bars. Average values accompanied by different letters (a, b, c) denote significant differences ($p < 0.05$) as a result of the dipping medium applied. No letters are included when significant differences were not found ($p > 0.05$). Initial fish value: 2.00 ± 0.00 log CFU g^{-1} muscle.

709 **Figure 3**: Effect of various dipping conditions* on the trimethylamine-N content (mg
710 kg^{-1} muscle) in megrim muscle throughout subsequent chilled storage**
711
712 * Abbreviations of dipping conditions as expressed in Table 1.
713 ** Average values of three (n = 3) replicates; standard deviations are indicated by bars.
714 Average values accompanied by different letters (a, b) denote significant
715 differences ($p < 0.05$) as a result of the dipping medium applied. No letters are
716 included when significant differences were not found ($p > 0.05$). Initial fish
717 value: 2.85 ± 1.10 mg kg^{-1} muscle.
718
719
720 **Figure 4**: Effect of various dipping conditions* on the sensory acceptability of megrim
721 muscle throughout subsequent chilled storage**
722
723 * Abbreviations of dipping conditions as expressed in Table 1.
724 ** Average values of three (n = 3) replicates; standard deviations are indicated by bars.
725 Average values accompanied by different letters (a, b) denote significant differences (p
726 < 0.05) as a result of the dipping medium applied. No letters are included when
727 significant differences were not found ($p > 0.05$). Initial fish value: 7.0 ± 0.0.
728

TABLE 1

Effect of the dipping treatment* including alga extract on various microbial groups (log CFU g^{-1} muscle) in megrim muscle throughout chilled storage**

Microbial parameter	Chilling time (days)	ND	DC	LAC	HAC
Aerobes	2	4.40 ab (1.11)	4.23 b (0.17)	3.77 a (0.20)	4.08 ab (0.36)
	6	5.16 (0.60)	4.55 (0.82)	4.42 (0.51)	4.58 (0.20)
	9	5.21 b (0.23)	4.80 ab (0.27)	4.70 a (0.02)	5.01 ab (0.48)
	13	7.10 b (0.29)	6.62 ab (0.81)	6.87 ab (0.42)	6.47 a (0.17)
Psychrotrophes	2	4.64 (0.60)	4.22 (0.33)	4.06 (0.26)	4.56 (0.34)
	6	5.79 b (0.72)	5.55 b (0.32)	5.39 b (0.26)	4.75 a (0.25)
	9	6.70 d (0.05)	6.31 bc (0.20)	5.58 a (0.49)	6.09 ab (0.20)
	13	7.48 (0.48)	6.91 (0.54)	7.07 (0.30)	6.84 (0.26)
Proteolytics	2	3.83 c (0.22)	3.43 ab (0.31)	2.82 a (0.36)	3.15 ab (0.32)
	6	5.25 b (0.54)	4.70 b (0.20)	4.45 b (0.29)	3.48 a (0.37)
	9	5.53 bc (0.42)	4.83 a (0.21)	4.69 ab (0.48)	5.69 c (0.40)
	13	6.91 (0.54)	6.32 (0.34)	6.61 (0.38)	6.56 (0.29)

* Abbreviations of dipping conditions: ND (without dipping; blank control), DC (dipping control), LAC (low-concentrated alga extract dipping) and HAC (high-concentrated alga extract dipping).

** Average values of three (n = 3) replicates; standard deviations are indicated in brackets. For each microbial group, average values followed by different letters (a, b, c, d) denote significant differences ($p < 0.05$) as a result of the dipping medium applied. No letters are included when significant differences were not found ($p > 0.05$). Initial fish values (log CFU g^{-1} muscle): 3.93±0.58 (aerobes), 3.65±0.17 (psychrotrophes), and 2.37±1.01 (proteolytics).

TABLE 2

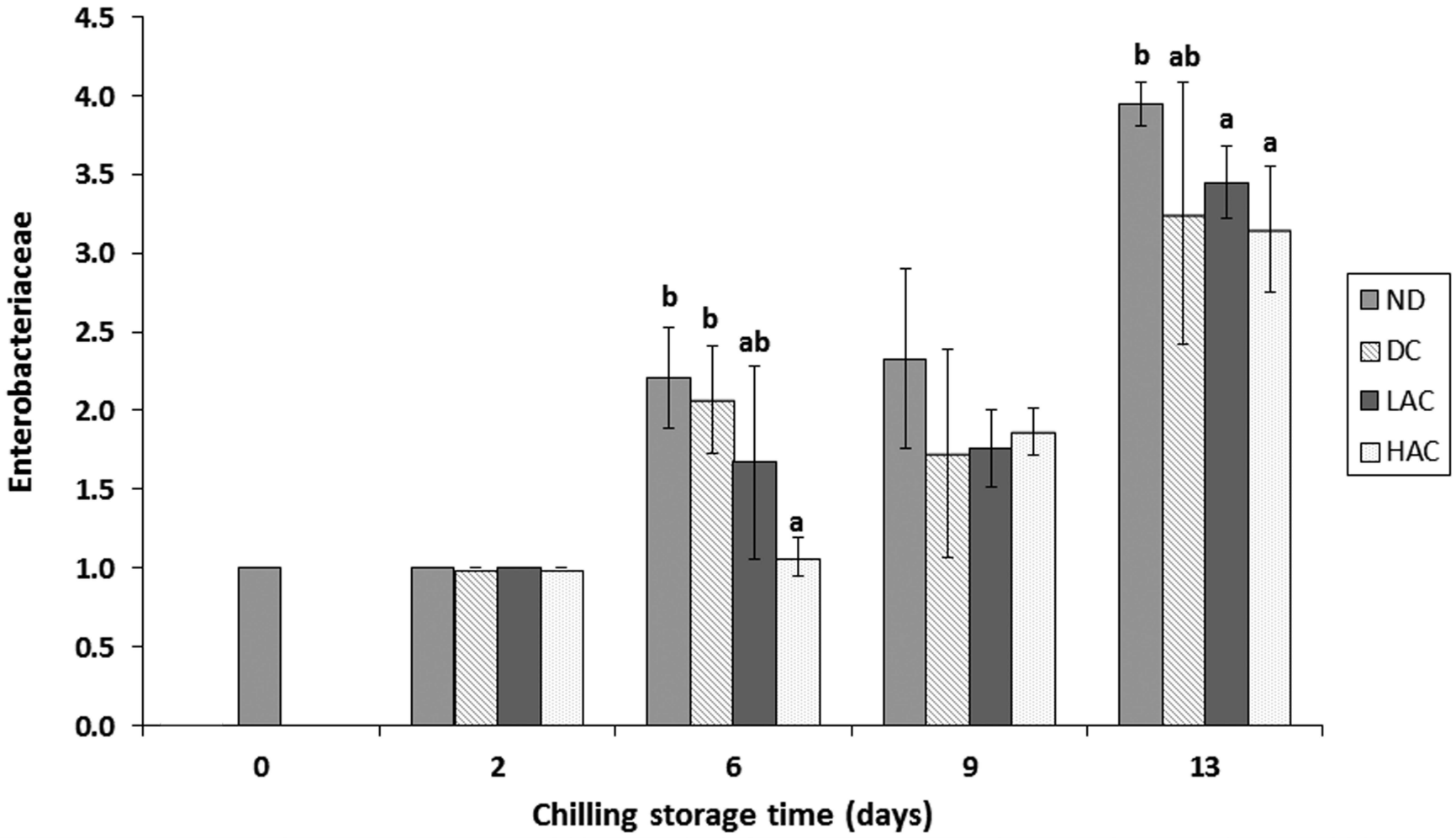
Effect of the dipping treatment* including alga extract on various chemical parameters in megrim muscle throughout chilled storage**

Chemical parameter	Chilling time (days)	ND	DC	LAC	HAC
pH	2	6.79 (0.17)	6.74 (0.12)	6.74 (0.03)	6.74 (0.03)
	6	7.04 (0.27)	6.97 (0.19)	6.84 (0.06)	6.87 (0.15)
	9	7.45 (0.29)	7.09 (0.10)	7.18 (0.12)	7.21 (0.05)
	13	7.49 b (0.22)	7.12 ab (0.20)	7.21 ab (0.11)	7.11 a (0.09)
Free fatty acids (mg kg^{-1} muscle)	2	21.41 ab (9.37)	21.05 b (4.44)	14.27 a (0.24)	18.93 ab (3.67)
	6	46.45 (10.22)	49.13 (16.93)	48.81 (28.79)	30.00 (5.84)
	9	65.03 (12.38)	62.27 (7.03)	51.68 (3.97)	41.73 (15.94)
	13	89.20 b (21.31)	88.72 b (17.09)	71.71 ab (27.44)	51.66 a (15.55)
Peroxide value (meq active oxygen kg^{-1} lipids)	2	1.22 a (0.26)	1.61 ab (0.43)	1.79 b (0.14)	2.15 b (0.33)
	6	1.54 (0.70)	1.39 (0.41)	1.58 (0.14)	1.99 (0.54)
	9	1.40 a (0.34)	1.90 ab (0.96)	2.21 ab (0.78)	2.44 b (0.46)
	13	2.20 (0.79)	2.46 (0.50)	2.24 (0.44)	2.53 (0.10)
Thiobarbituric acid index (mg malondialdehyde kg^{-1} muscle)	2	0.44 (0.15)	0.36 (0.08)	0.18 (0.09)	0.29 (0.05)
	6	0.38 ab (0.17)	0.38 b (0.01)	0.29 a (0.01)	0.32 ab (0.06)
	9	0.36 (0.04)	0.45 (0.05)	0.51 (0.09)	0.35 (0.07)
	13	0.40 (0.07)	0.39 (0.07)	0.37 (0.06)	0.32 (0.03)

* Abbreviations of dipping conditions as expressed in Table 1.

** Average values of three (n = 3) replicates; standard deviations are indicated in brackets. For each chemical parameter, average values followed by different letters (a, b) denote significant differences ($p < 0.05$) as a result of the dipping medium applied. No letters are included when significant differences were not found ($p > 0.05$). Initial fish values: 6.57±0.12 (pH), 9.09±1.15 mg kg^{-1} muscle (free fatty acids), 0.80±0.05 meq active oxygen kg^{-1} lipids (peroxide value), 0.31±0.06 mg malondialdehyde kg^{-1} muscle (thiobarbituric acid index).

Figure 1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Chilling storage time (days)
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DC
LAC
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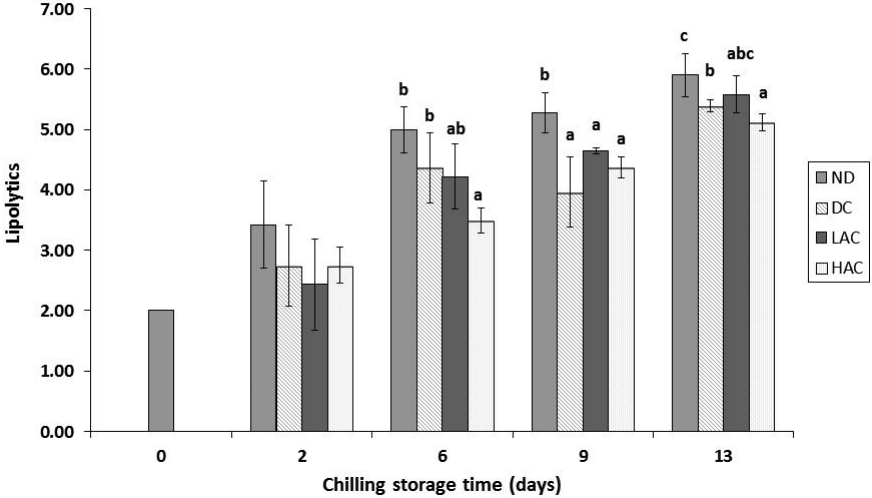
Figure 2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Chilling storage time (days)
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LAC
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Figure 3

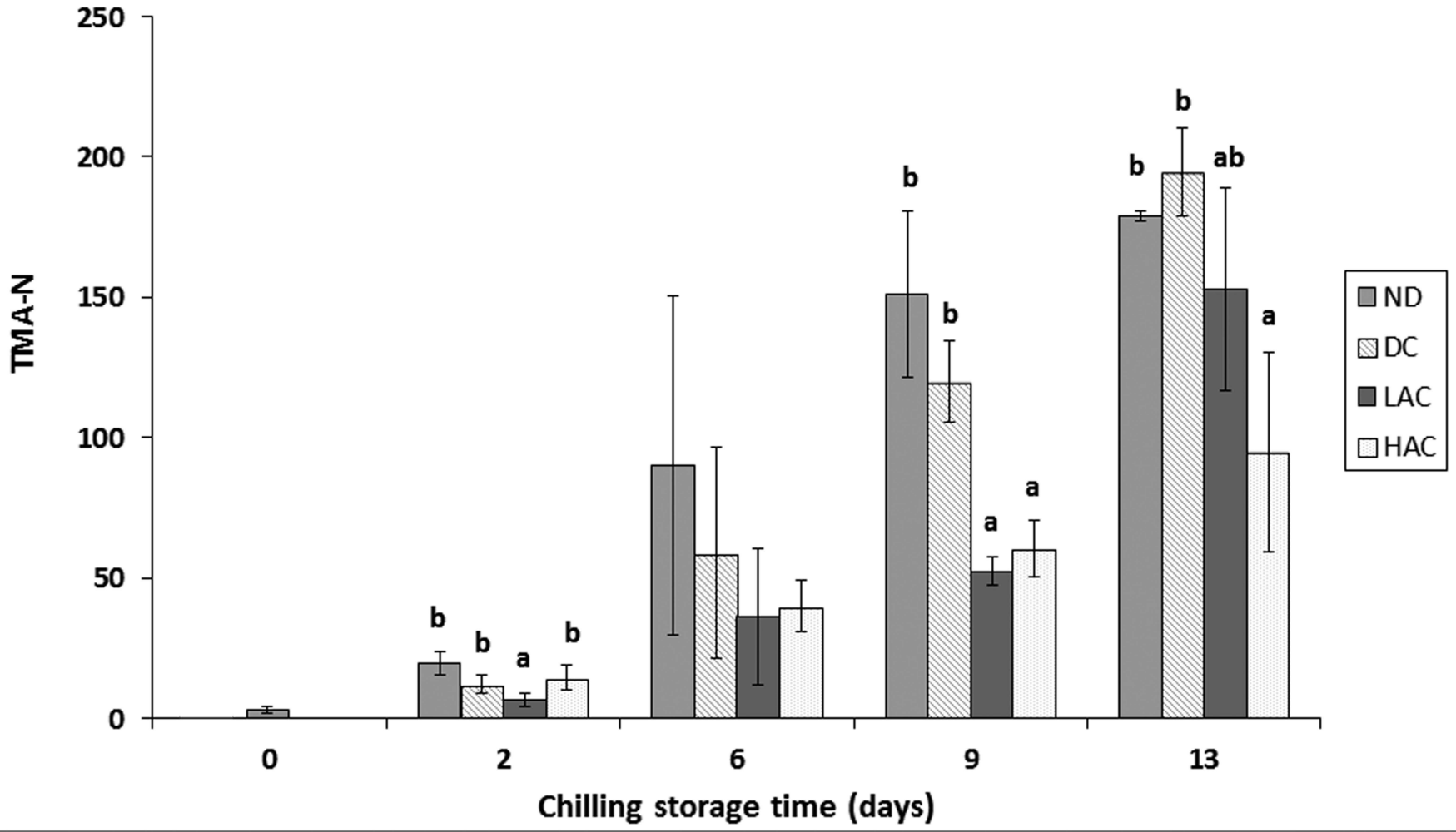
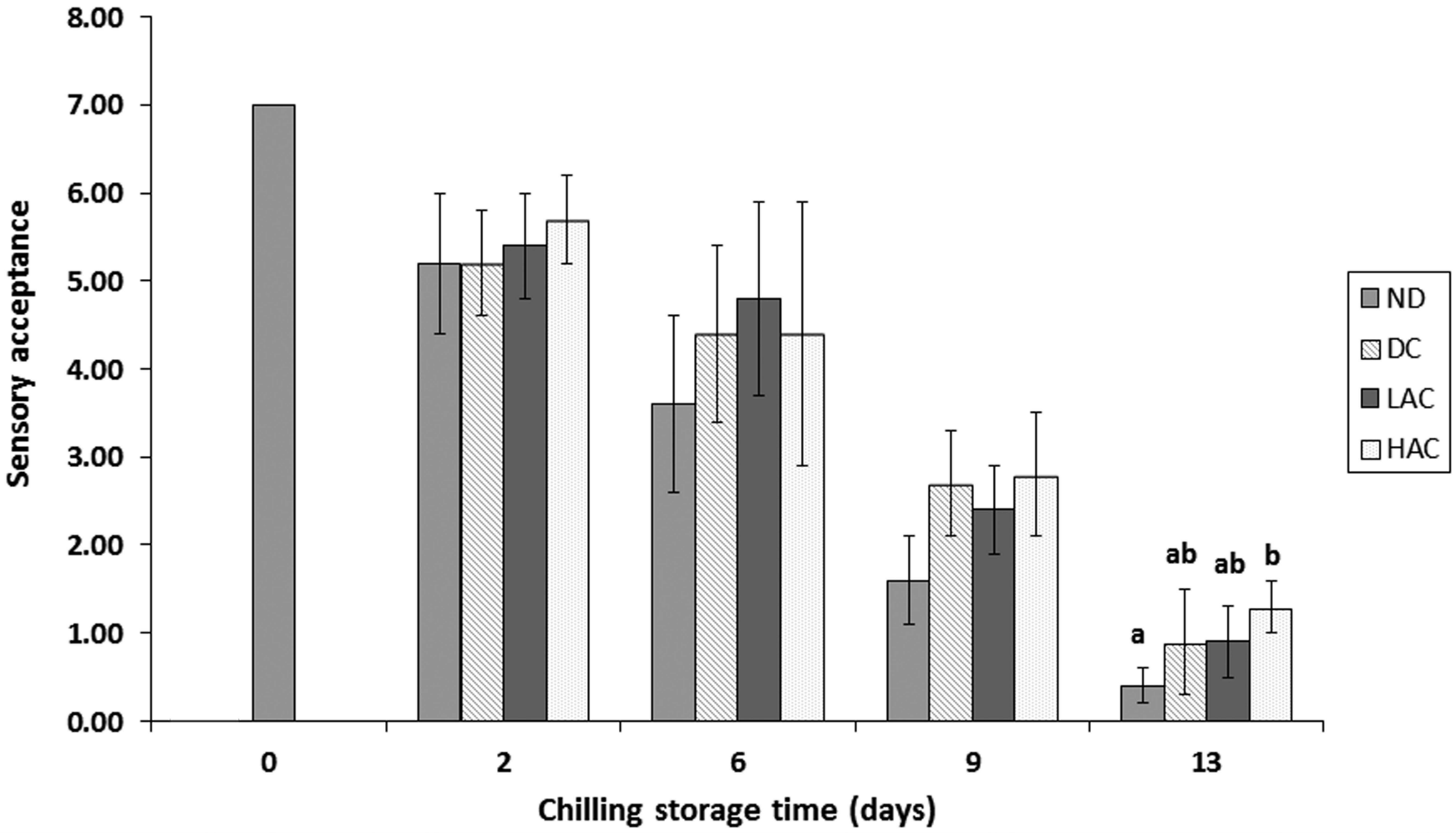

250
200
150
100
50
0
TMA-N
b
b
a
b
b
b
a
a
b
b
ab
a
0
2
6
9
13
Chilling storage time (days)
ND
DC
LAC
HAC

Figure 4


8.00
7.00
6.00
5.00
4.00
3.00
2.00
1.00
0.00
Sensory acceptance
0
2
6
9
13
Chilling storage time (days)
a
ab
ab
b
ND
DC
LAC
HAC