Effect of biodegradable film (lyophilised alga *Fucus spiralis* and sorbic acid) on quality properties of refrigerated megrim (*Lepidorhombus whiffiagonis*)

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SUMMARY

The effect of replacing the on-board currently employed polyethylene film by a novel type of environmentally-friendly packaging was studied. For it, a polylactic acid (PLA) biodegradable film including lyophilised alga *Fucus spiralis* and sorbic acid was applied during megrim (*Lepidorhombus whiffiagonis*) refrigeration and its effect on fish quality loss was evaluated. Thus, sensory assessment showed that samples wrapped up with PLA film including 8% alga and 1% sorbic acid were still acceptable on day 11, while control fish specimens (kept under polyethylene film) were rejected at that time. Under such biodegradable film condition, a preservative effect was also implied according to chemical indices assessment related to microbial activity (trimethylamine-N) and lipid oxidation development (peroxide and fluorescent compounds formation); additionally, lower mean numbers for different microbiological groups (aerobes, Enterobacteriaceae and psychrotrophs) were detected. This result provides a promising replacement strategy to enhance refrigerated fish quality and reduce the waste material content.

Keywords: *Lepidorhombus whiffiagonis*; refrigeration; biodegradable film; sorbic acid; *Fucus spiralis*; quality.

Running title: Biodegradable film and megrim refrigeration.

INTRODUCTION

For years, refrigerated fish has shown to dominate the markets and represent very high proportions in fish production and human consumption, being flake-ice chilling the most commonly used method. However, deterioration of sensory quality and nutritional value occurs in refrigerated fish as a result of microbial and biochemical degradation mechanisms. This fast degradation can be explained on the basis that seafood arise from poikilothermic organisms with both a high water and non-protein nitrogen content, a soft muscular and skin structure, a low collagen content and a highly unsaturated lipid composition (Campos *et al.*, 2012). According to the actual need for high-quality fresh products, flake ice has been combined with other preservative strategies in order to enlarge the shelf life of the refrigerated products (Ashie *et al.*, 1996; Sivertsvik *et al.*, 2002).

One of such strategies has been the employment of natural low molecular weight organic acids. Such compounds represent a relevant choice because of their easy availability, low commercial cost and wide range of permitted concentration for use (Madrid *et al.*, 1994; Sanjuás-Rey *et al.*, 2012). Among them, sorbic acid and its salts have shown a marked preservative activity, this leading to a higher freshness and quality retention in refrigerated fish products (Erkan *et al.*, 2001; Yesudhason *et al.*, 2010). On the other side, natural products of marine origin such as red, green and brown macroalgae have offered the possibility of exploring a wide variety of natural compounds with potential antioxidant and antimicrobial activity (Wang *et al.*, 2010; Halldorsdóttir *et al.*, 2014). Additionally, macroalgae are known to be part of the diet in different countries and constitute a source of beneficial nutrients, such as vitamins, trace minerals, lipids, amino acids and dietary fibres (Díaz-Rubio *et al.*, 2009; Paiva *et al.*, 2014).

During on-board storage, unloading and retail distribution, fish specimens are often wrapped up with plastic films to avoid direct contact with extra-cold ice that may lead to an important surface damage. These films are known to be made of synthetic polymers such as polyethylene and can constitute great quantities of non-degradable waste material in harbours and related locations during the distribution chain. The replacement of such synthetic films by biodegradable ones including preservative compounds constitutes a promising strategy to be applied to refrigerated fish (López-Rubio *et al.*, 2006). In this sense, polylactic acid (PLA) is increasingly being adopted as a biodegradable thermoplastic polyester for packaging application (Sansone *et al.*, 2012; Fortunaty *et al.*, 2012). Additionally, different kinds of preservative compounds have been introduced into the PLA-packaging system in order to increase the antimicrobial (nisin, EDTA, sodium benzoate, potassium sorbate) (Jin *et al.*, 2010) and antioxidant (ascorbyl palmitate, α -tocopherol) (Jamshidian *et al.*, 2012) properties.

The present research focuses on the quality retention of megrim (*Lepidorhombus whiffiagonis*) during refrigerated storage. This flatfish species is considered one of the most fished species in the Grand Sole North Atlantic Fishing bank, and has been exploited by a wide number of European countries, including the United Kingdom, France, Ireland and Spain. During its commercialisation, most problems have shown to be related to the time elapsed between catching and arrival to ultimate destination. Thus, previous research on megrim has demonstrated that the quality of this species diminishes during on-board storage, so that different preservative technologies have been tested during such storage period (Aubourg *et al.*, 2006; Pastoriza *et al.*, 2008; García-Soto *et al.*, 2014).

The objective of the current work was to study the effect of replacing the on-board currently employed polyethylene film by a novel type of environmentally-

friendly packaging. For it, a PLA film including sorbic acid and lyophilised alga *Fucus spiralis* was applied and its effect on sensory, microbiological and chemical parameters related to quality loss in refrigerated megrim was analysed. *Fucus spiralis* was chosen for this study in agreement with its abundance in the Galician Atlantic coast (northwestern Spain) and its promising preserving properties revealed in recent studies (Andrade *et al.*, 2013; Tierney *et al.*, 2013a).

MATERIAL AND METHODS

Film systems preparation

Commercial PLA (Bio-Flex® F 6510, FKuR Kunststoff GmbH; Willich, Germany) with a melt flow index (MFI) of 4.3 (± 0.2) g 10⁻¹ min⁻¹ (190°C, 2.16 kg) and a density of 1.29 (± 0.01) g cm⁻³ was used. Lyophilised alga (*Fucus spiralis*) was provided by Porto-Muiños (Cereda, A Coruña, Spain) and incorporated into the biodegradable film at a concentration of 8% (w/w, alga/PLA). Sorbic acid (Merck, Darmstadt, Germany) was incorporated at two different concentrations (w/w, sorbic acid/PLA): 0.5% (S-0.5 film condition) and 1.0% (S-1 film condition).

Biodegradable films based on PLA were obtained by means of an extrusion process. Both sorbic acid and lyophilised alga were incorporated during the masterbatch preparation. For it, a Brabender DSE-20 twin-screw extruder (Duisburg, Germany) with a flat die was used to process the films; barrel and die temperature were about 160-170°C and the screw speed was 20 rpm. The melted polymer formulations were cooled on a chilled roll and stretched in the machine direction. The chilled roll was kept at 80 $\pm$ 1°C with a rotation speed of 1.4 rpm. The thickness of the films was measured with a hand-held micrometre. The average values were 357 and 300 μ m for S-0.5 and S-1 film batches, respectively.

A polyethylene film was employed as control (C film condition). It was manufactured in an industrial extruder and exhibited a film thickness of 150 μm .

Experimental conditions (percentages of sorbic acid and alga extract) employed in the present study are based on several preliminary tests carried out in our laboratory. Thus, sorbic acid employment was tested in a 0.2-2.0% range. It could be observed that a higher presence than 1% might damage some sensory descriptors (namely, eyes and skin); accordingly, 0.5% and 1.0% were chosen for the actual research. Sorbic acid is a “generally recognized as safe” (GRAS) natural substance for use in food technology according to European and American standards (Madrid et al., 1994; Giese, 1996).

Concerning the alga extract, an increasing presence in the biodegradable film showed to infer better sensory acceptance; thus, 8% content was chosen as being the highest concentration allowed for the preparation of the actual biodegradable film. According to European Council Regulation (1997), algae are considered food or food ingredients; from the food safety point of view, their use in the biodegradable films should not constitute any hazard to health.

Fish material, processing and sampling

Fresh megrim (*Lepidorhombus whiffiagonis*) (120 specimens) were caught near the Galician Atlantic coast (north-western Spain) and transported to the laboratory. Throughout this process (10 h), the fish were maintained in ice. The length and weight of the fish specimens ranged from 21 to 24 cm and from 97 to 125 g, respectively.

Upon arrival to the laboratory, 12 individual fish specimens were separated and analysed as initial material (day 0). These fish specimens were divided into three different groups (four individuals per group) that were analysed independently to achieve the statistical analysis ($n=3$). The remaining fish specimens were divided into

three batches (36 individuals in each batch), that were placed in independent boxes and wrapped up with different kinds of films (C, S-0.5 and S-1, respectively), prepared as previously described. Then, ice was added at a 1:1 fish/ice ratio, so that the film avoided the direct contact of fish with ice; all batches were placed inside a refrigerated room ($4\pm1^{\circ}\text{C}$). Boxes that allowed draining of melted ice were used for fish storage. The ice of all batches was renewed when required. Fish samples from all of the batches were stored for a 11-day period, being sampled and analysed on days 4, 7 and 11. At each sampling time, 12 specimens were taken from each batch for analysis and divided into three groups (four individuals in each group) that were studied independently ($n=3$). Sensory analysis was carried out on the whole fish, whereas microbiological and chemical analyses were carried out on the white muscle.

Microbiological analyses

Samples of 10 g of fish muscle were dissected aseptically from refrigerated fish specimens, mixed with 90 mL of 0.1% peptone water (Merck, Darmstadt, Germany) and homogenised in sterilised stomacher bags (AES, Combourg, France) as previously described (Ben-Gigirey *et al.*, 1998; Ben-Gigirey *et al.*, 1999). In all of the cases, serial dilutions from the microbial extracts were prepared in 0.1% peptone water.

Total aerobes were investigated by surface inoculation on plate count agar (PCA, Oxoid Ltd., London, UK) after incubation at 30°C for 48 h. The anaerobe counts were also determined in PCA at $30\pm0.5^{\circ}\text{C}$, except that an anaerobic atmosphere kit (Oxoid Ltd.) was placed together with the plates inside the anaerobiosis jar. Psychrotrophs were also investigated in PCA, being the incubation carried out at $7-8^{\circ}\text{C}$ for 7 days. Enterobacteriaceae were investigated via pour plating using Violet Red Bile Agar (VRBA) (Merck, Darmstadt, Germany) after incubation at $37\pm0.5^{\circ}\text{C}$ for 24 h. In

all of the cases, bacterial counts were transformed into log CFU g⁻¹ muscle before undergoing statistical analysis. All of the analyses were conducted in triplicate.

Chemical analyses

Total polyphenols content of lyophilised *Fucus spiralis* was assessed by means of the Folin-Ciocalteu colorimetric method (Cary 3E UV–Visible spectrophotometer, Varian; Mulgrave, Victoria, Australia) as described previously (Rodríguez-Bernaldo de Quirós *et al.*, 2010). Measurements were made in triplicate. Gallic acid (GA) was used as standard. Results were expressed as mg GA g⁻¹ lyophilised alga.

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

Trimethylamine-nitrogen (TMA-N) values were determined using the picrate colorimetric (Beckman Coulter, DU 640; London, UK) method, as previously described by Tozawa *et al.*, (1971). This method involves the preparation of a 5% trichloroacetic acid extract of fish muscle (10 g/25 mL). The results are expressed as mg TMA-N kg⁻¹ muscle.

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

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

Tertiary lipid oxidation compounds resulting from the interaction between oxidised lipids and nucleophilic compounds (namely, protein-like molecules) were

measured by fluorescence spectroscopy. Such measurement has shown to be a valuable tool for assessing the fish quality changes during processing (Aubourg, 1999b). The formation of fluorescent compounds (Fluorimeter LS 45; Perkin Elmer España; Tres Cantos, Madrid, Spain) was determined by measurements at 393/463 nm and 327/415 nm as described by Aubourg (1999a). The relative fluorescence (RF) was calculated as follows: $RF = F/F_{st}$, where F is the fluorescence measured at each excitation/emission maximum and F_{st} is the fluorescence intensity of a quinine sulphate solution ($1 \mu\text{g mL}^{-1}$ in $0.05 \text{ M H}_2\text{SO}_4$) at the corresponding wavelength. The fluorescence ratio (FR) was calculated as the ratio between the two RF values: $FR = RF_{393/463 \text{ nm}}/RF_{327/415 \text{ nm}}$. The FR value was determined using the aqueous phase that resulted from the lipid extraction of the fish muscle (Bligh & Dyer, 1959).

All solvents and chemical reagents used were of reagent grade (Merck, Darmstadt, Germany).

Sensory analysis

Sensory analysis was conducted by a sensory panel that consisted of five experienced judges who adhered to traditional guidelines concerning fresh and refrigerated fish, which was adapted to megrim (European Council Regulation, 1996). The panellists had participated in the sensory analysis of various fish and seafood products for the previous 15 years. Before carrying out the present experiment, the judges received special training on refrigerated megrim, focused on the evaluation of refrigerated specimens that exhibited different qualities. Special attention was paid to the evolution of the sensory descriptors that were found as limiting factors for the shelf life.

Four categories were ranked (European Council Regulation, 1996): highest quality (E), good quality (A), fair quality (B) and unacceptable quality (C), according to

Table 1. Sensory assessment of the fish included the following descriptors: skin and mucus development, eyes, external odour, gill appearance and odour, consistency, flesh (raw and cooked) odour and flesh (cooked) taste. Sensory evaluation began by the analysis of fish in the raw state and was followed by the analysis in the cooked state. Cooking was accomplished at 95-100°C for 7 min in a pre-warmed oven with air circulation and then submitted to the panel. At each sampling time, whole fish specimens were coded with 3-digit random numbers and presented to the panellists in individual trays, which were scored individually. Each descriptor of each sample was scored a single time by each member of the panel. The panel members shared samples tested.

Statistical analysis

Data obtained from the different microbiological and chemical analyses were subjected to the ANOVA method to explore differences resulting from the effects of both the film condition and the refrigeration time; the comparison of means was performed using the least-squares difference (LSD) method. Data obtained from the sensory evaluation were analysed by the non-parametric Kruskal-Wallis test. In all cases, analyses were carried out using the PASW Statistics 18 software for Windows (SPSS Inc., Chicago, IL, USA); differences among batches and among refrigeration times were considered significant for a confidence interval at the 95% level ($p < 0.05$) in all cases.

RESULTS AND DISCUSSION

Quality loss assessment by microbiological analysis

The evolution of aerobic mesophiles in megrim is displayed in Figure 1. No statistically significant ($p > 0.05$) differences were observed between the control batch (C) and any of

the batches that included a biodegradable film. It is concluded that the present preserving conditions (S-0.5 and S-1) were not found useful to provide an activity decrease in this microbiological group. However, the contact of the fish surface with the biodegradable film harbouring alga extract and sorbic acid in S-0.5 and S-1 batches implied a slight reduction of the mean aerobe numbers, the highest difference being observed between C and S-1 batches after 7 days of storage ($0.80 \log \text{CFU g}^{-1}$). In this sense, the release of anti-microbial compounds from the film towards the fish flesh was not so intense as to provide significant ($p < 0.05$) differences in the development of aerobic mesophiles among batches. This limited effect might be explained by the fact that only one side of the fish was in contact with the film. It should also be remarked that the microbial quality of the fish, in terms of aerobic mesophile counts, was good, the batches exhibiting microbial loads well below 6 log units even after seven days of storage. This overall good microbial quality of the fish material may also explain the little differences observed among batches.

The investigation of Enterobacteriaceae (Figure 2) provided similar results to aerobe counts, although the differences between mean numbers of S-1 and C batches were higher in the case of Enterobacteriaceae. Thus, lower mean counts were determined for this microbial group at all storage times in the S-0.5 batch and, especially in S-1 batch as compared to the control batch.

The evolution of total psychrotrophs is expressed in Table 2. Such group is mainly composed of Gram-negative rod-shaped bacteria belonging to the genera *Pseudomonas*, *Moraxella*, *Acinetobacter*, *Shewanella* and *Flavobacterium*. Psychrotroph counts did not reveal any significant ($p > 0.05$) differences among batches throughout storage (Table 2). However, remarkable lower mean values were observed on days 7 and 11 in samples corresponding to PLA-based biodegradable films, as

compared to C batch. Thus, the greatest mean contents difference was obtained between C and S-1 batches on day 7 ($0.90 \log \text{CFU g}^{-1}$), a result that indicated that the contact of the megrim surface with the alga-sorbic acid film was likely to provide some protection against microbes in the megrim muscle.

The investigation of anaerobe counts in megrim muscle evidenced that this microbial group did not exhibit significant ($p>0.05$) differences among batches at any storage time (Table 2). It should be remarked that none of the batches exhibited anaerobe counts above $3 \log \text{CFU g}^{-1}$, even after 11 days of refrigerated storage. Thus, and in contrast with the results obtained for aerobic mesophiles, Enterobacteriaceae and psychrotrophs, no remarkable effect of the films containing alga and sorbic acid on the mean numbers of anaerobes was achieved.

Although a significant effect on the different microbial groups tested could not be obtained ($p>0.05$) in the present experimental conditions, some profitable effect of the biodegradable films employment could be implied as a result of the reduction of the mean numbers in most microbiological groups; this effect could be observed in psychrotrophe (7-11-day period), aerobe (day 7) and Enterobacteriaceae (4-11-day period) counts. The antimicrobial effects observed in this study are a consequence of the incorporation of sorbic acid and *Fucus spiralis* extract to the films. Thus, sorbic acid is an approved food additive whose preservative effect on foods is based on the inhibition of microbial key metabolic pathways (York & Vaughn, 1964). The anti-microbial effect of *Fucus* spp. and other algae have been reported to be caused by terpenes and polyphenols (Sandsalen *et al.*, 2003), among other compounds. Thus, previous plate bioassays carried out on our laboratory showed that ethanolic extracts of *Fucus spiralis* exhibited antimicrobial activity against *Bacillus cereus*, *Bacillus subtilis*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Pseudomonas fluorescens*,

Escherichia coli, *Aeromonas hydrophila*, *Vibrio alginolyticus* and *Vibrio parahaemolyticus* (data not shown).

This study performed on megrim complements other previous reports focused on the preservation of this fish species under advanced refrigeration systems, such as on-board employment of slurry ice (Aubourg *et al.*, 2006) and addition of ozone to flake ice (Pastoriza *et al.*, 2008). Other previous studies have also reported benefits derived from the inclusion of citric acid and lactic acid in flake ice used for megrim storage (García-Soto *et al.*, 2014). Moreover, the inclusion of ascorbic acid, together with citric and lactic acids has also proved to help in the control of aerobic mesophiles in megrim as well as in hake (*Merluccius merluccius*) and angler (*Lophius piscatorius*) (Sanjuás-Rey *et al.*, 2012).

Quality loss assessment by chemical analysis

A marked pH increase ($p < 0.05$) with refrigeration time was observed in all kinds of samples (Table 2). On day 11, all samples exhibited pH values above 7.0, this revealing a significant quality loss. Comparison among different batches did not provide significant differences ($p > 0.05$), although higher mean values were observed in fish specimens corresponding to control batch in the 7-11-day period.

Increase in the pH of the fish muscle indicates the accumulation of alkaline value compounds, such as ammonia compounds and TMA, which are principally derived from microbial activity. In this sense, it has been suggested that pH values above 7.0 may limit the shelf life of certain fish species such as hake (Ruiz-Capillas & Moral, 2001). However, previous research related to megrim refrigerated storage has shown that this species could still be considered as acceptable by the sensory panel with pH values slightly above 7.0 (Aubourg *et al.*, 2006; Pastoriza *et al.*, 2008).

The antimicrobial activity of sorbic acid has shown to increase as the pH of the substrate decreases and approaches the dissociation constant ($pK_a=4.76$, 25°C) (Sofos, 2000); thus, the inhibitory action of the undissociated acid has been reported to be greater than that of the dissociated acid (Eklund, 1983). However, sorbic acid has shown effectiveness at pH values round 6.5, while certain studies have indicated small antimicrobial activity at pH values as high as 7.0 (Statham & McMeekin, 1988; Sofos, 2000). Concerning fish-based food, sorbic acid has shown a profitable antimicrobial effect when applied to smoked blue catfish (*Ictalurus furcatus*) steaks (Antonia da Silva et al., 2008), Chub mackerel (*Trachurus murphyi*) fillets (Erkan et al., 2001), and seer fish (*Scomberomorus commerson*) steaks (Yesudhasan et al., 2010); in such cases, the pH value was included in the 6.0-6.7 range, in agreement with the present research for the 0-7-day period; contrary, actual pH values resulting at the end of the experiment (7.13-7.23 range) would correspond to a non-profitable environment for the sorbic acid preservative action.

Increasing TMA-N values ($p<0.05$) were obtained as refrigeration time progressed in all fish batches (Table 2). At the end of the experiment, fish samples corresponding to C and S-0.5 batches overpassed the 120.0-mg kg^{-1} score. Remarkably, at that time an inhibitory effect ($p<0.05$) on TMA formation could be concluded as a result of applying the biodegradable PLA-based films including alga and sorbic acid. This effect was more intense when the highest sorbic acid content was present in the film.

Volatile amine compounds are partially produced by means of endogenous enzyme activity but mostly as a result of microbial development (Campos *et al.*, 2012). As in the current study, previous studies have shown a high TMA formation in megrim muscle during chilled storage (Civera *et al.*, 1995; Aubourg *et al.*, 2006; Sanjuás-Rey *et*

al., 2012). It is worth of pointing out that megrim samples corresponding to S-1 batch did not reach its legal limit (120 mg kg^{-1}) (European Council Directive, 1991), while the other two batches overpassed such limit. TMA formation in refrigerated megrim has also been reported to be inhibited as a result of applying other preservative strategies such as slurry ice (Aubourg *et al.*, 2006) and ozonised ice (Pastoriza *et al.*, 2008).

Formation of peroxides was found to be relevant on day 4 for all kinds of samples (Table 3). After that time, only a slight increase was observed in general till the end of the experiment. In all cases, PV remained below a concentration of 7.23 meq kg^{-1} lipids, this indicating a relatively low peroxide formation in all batches (Aubourg, 1999a), according to previous literature related to refrigerated megrim (García-Soto *et al.*, 2011). However, no significant differences ($p>0.05$) among batches derived from the use of PLA-based films could be concluded for the PV; additionally, mean values were found very similar among batches.

Formation of fluorescent compounds was also found to be very low (Table 3), taking into account the initial fish value. No significant effect ($p>0.05$) of the refrigeration time was observed for any of the batches under analysis. However, a higher FR score ($p<0.05$) was observed on day 7 in control fish when compared with both PLA-based batches, this reflecting a protective effect of the biodegradable films under study; additionally, higher mean values were present in control samples at the end of the experiment.

Lipid oxidation is not considered a major damage pathway during refrigerated storage of lean fish (lipid content range in the present experiment: $4.2\text{-}5.3 \text{ g kg}^{-1}$ megrim muscle) and the present study confirmed that there was a low primary and tertiary lipid oxidation development, as previously reported (Sanjuás-Rey *et al.*, 2011; Campos *et al.*,

2012). Interestingly, the control batch exhibited the highest FR value on day 7, which suggests a significant protective effect of the biodegradable films tested on fish quality.

This antioxidant effect of biodegradable films can be explained on the basis of the presence of polyphenol compounds in ethanolic extracts (53.3 ± 5.0 mg GA g⁻¹) of alga *Fucus spiralis* facilitated by the fact that PLA-packaging system has shown a valuable release of phenolic antioxidants from the extruded film (Jamshidian *et al.*, 2012). This antioxidant capacity of ethanolic extracts of alga *Fucus spiralis* was already proved in different in-vitro tests (Andrade *et al.*, 2013), showing a marked content on polyphenols (90-205 µg phloroglucinol equivalents mg⁻¹) (Tierney *et al.*, 2013a) and α-tocopherol (511.4 mg kg⁻¹) (Paiva *et al.*, 2014). Additionally, a preliminary identification of active compounds was carried out (Tierney *et al.*, 2013b); thus, analysis by quadrupole time-of-flight mass spectrometry (Q-Tof-MS) supported the assumption that phlorotannins were present and likely to be responsible for the observed antioxidant activities.

Previous research had shown that other strategies also led to an inhibitory effect on lipid oxidation development in refrigerated megrim. Thus, the employment of a commercial formula including citric, lactic and ascorbic acids led to a slower lipid oxidation development during the chilled storage of several species such as megrim, hake and angler (García-Soto *et al.*, 2011).

Quality loss assessment by sensory analysis

Evaluation of the different sensory parameters was carried out for all types of batches and the results are compiled in Table 4. Sensory score decreased as refrigeration time progressed in all batches. The control batch was found to be unacceptable on day 11, whereas fish corresponding to the S-1 batch was still acceptable at that time. Thus, an

inhibitory effect of the PLA-based biodegradable films on sensory quality loss could be concluded. Limiting sensory parameters were external odour, gill appearance and odour, and flesh odour and taste. There were no differences in the skin and mucus development, eyes appearance and consistency among fish corresponding to the different batches.

Concerning fish corresponding to S-0.5 batch, some sensory features were better maintained (external odour, gill appearance and odour) when compared with control batch. However, samples belonging to the S-0.5 batch were found to be unacceptable by the panel on day 11, according to the evaluation of flesh odour and taste.

This increase of sensory acceptance and shelf life for fish stored under biodegradable film conditions is in agreement with the above-mentioned results concerning microbiological and chemical assessments. Thus, under S-1 biodegradable film condition, a preservative effect ($p < 0.05$) was also implied according to chemical indices assessment related to microbial activity (TMA-N) and lipid oxidation development (PV and FR); additionally, a lower mean microbial development was detected according to the measurement of aerobe, Enterobacteriaceae and psychrotroph counts.

Previous research demonstrated an increased shelf life and a sensory quality enhancement in chilled megrim by means of including preservative compounds in the icing system. This is the case with ozone during the on-board chilled storage of this species (Aubourg *et al.*, 2006; Pastoriza *et al.*, 2008). An increased shelf life of chilled megrim has also previously been observed as a result of including natural preservative compounds in the icing system (García-Soto *et al.*, 2014). Indeed, the shelf life of chilled megrim, hake and angler has been extended by applying a commercial formula

including citric, lactic and ascorbic acids in the icing medium (Sanjuás-Rey *et al.*, 2012).

CONCLUSIONS

The employment of a polylactic acid-based film including sorbic acid and lyophilised alga *Fucus spiralis* has shown a protective effect on refrigerated megrim quality. Thus, sensory assessment showed that samples wrapped up with polylactic-acid biodegradable film including 8% alga and 1% sorbic acid were still acceptable on day 11, while control fish specimens were rejected at that time; among limiting factors, external odour, gill appearance and odour and flesh odour and taste can be mentioned. Under such biodegradable film condition, a preservative effect ($p < 0.05$) was also implied according to chemical indices assessment related to microbial activity (trimethylamine-nitrogen) and lipid oxidation development (peroxide value and fluorescence ratio); additionally, a lower mean microbial development was detected according to the measurement of aerobe, Enterobacteriaceae and psychrotroph counts.

The present study provides a first approach focused on the replacement of the on-board currently employed polyethylene film by a novel type of environmentally-friendly packaging. The results presented in this study constitute a promising basis in order to enhance fish quality retention during commercial on-board long storage and to reduce the waste material content produced during the different steps included in the fish distribution chain, from on-board storage until retail distribution. Further research is envisaged concerning the optimisation of polylactic acid-based films application to marine species. In them, the particular role of each preserving component (i.e., lyophilised alga and sorbic acid) ought to be assessed, this including the possible synergistic or additive effect. Once the biodegradable film may be applied to the food

452 manufacture, analysis of any undesirable compound (namely, As), its release from the
453 film and its stability during the film extrusion process should be addressed.

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FIGURE LEGENDS

Figure 1: Aerobe count assessment* in refrigerated megrim wrapped up with different film conditions**

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

** Film conditions: C (polyethylene film; control), S-0.5 (polylactic-acid biodegradable film including 8% lyophilised alga and 0.5% sorbic acid) and S-1 (polylactic-acid biodegradable film including 8% lyophilised alga and 1.0% sorbic acid), according to the Material and Methods section. IN (initial fish; day 0).

Figure 2: Enterobacteriaceae count assessment* in refrigerated megrim wrapped up with different film conditions**

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

** Film conditions as expressed in Figure 1. IN (initial fish; day 0).

TABLE 1

Scale employed for evaluating the sensory quality of refrigerated megrim

Descriptor	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
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
External odour	Sharply seaweed and shellfish smell	Weakly seaweed and shellfish smell	Incipiently putrid or ammonia odour	Putrid or ammonia odour
Gills appearance and odour	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
Consistency	Presence of partial disappearance of rigor mortis symptoms	Firm and elastic; pressure signs disappear immediately and completely	Presence of mechanical signs; elasticity notably reduced	Important shape changes due to mechanical factors
Flesh odour (raw fish)	Sharply seaweed and shellfish	Weakly seaweed and shellfish	Incipiently putrid or ammonia odour	Putrid or ammonia odour
Flesh odour (cooked fish)	Sharply fresh and agreeable	Weakly fresh and agreeable	Incipiently putrid or ammonia odour	Putrid or ammonia odour
Flesh taste	Sharply fresh and agreeable	Weakly fresh and agreeable	Incipiently putrid or ammonia odour	Putrid or ammonia odour

TABLE 2

Microbial activity assessment* in refrigerated megrim wrapped up with different film conditions**

Quality index	Film condition	Refrigeration storage time (days)			
		0	4	7	11
Psychrotrophe counts (log CFU g ⁻¹ muscle)	C	3.20 a (1.06)	4.36 a (1.30)	6.57 ab (2.23)	7.62 b (2.54)
	S-0.5	3.20 a (1.06)	4.54 ab (1.34)	5.74 ab (1.77)	6.97 b (2.27)
	S-1	3.20 a (1.06)	4.44 ab (1.37)	5.67 ab (1.60)	7.23 b (2.37)
Anaerobe counts (log CFU g ⁻¹ muscle)	C	< 2.00	< 2.00	2.37 (1.09)	2.37 (1.02)
	S-0.5	< 2.00	< 2.00	2.20 (0.91)	2.55 (1.30)
	S-1	< 2.00	< 2.00	2.30 (0.65)	2.51 (1.25)
pH	C	6.41 a (0.04)	6.48 a (0.03)	6.70 b (0.12)	7.23 c (0.39)
	S-0.5	6.41 a (0.04)	6.51 ab (0.12)	6.62 b (0.19)	7.17 c (0.16)
	S-1	6.41 a (0.04)	6.55 ab (0.12)	6.68 b (0.12)	7.13 c (0.08)
Trimethylamine-N (mg kg ⁻¹ muscle)	C	1.7 a (0.1)	1.8 a (0.1)	13.5 b (1.4)	148.6 cC (16.8)
	S-0.5	1.7 a (0.1)	1.6 a (0.1)	23.4 b (11.6)	129.0 cB (5.2)
	S-1	1.7 a (0.1)	1.6 a (0.1)	18.1 b (6.4)	107.1 cA (13.7)

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

For each parameter and for each refrigeration time, mean values followed by different capital letters (A-C) indicate significant differences (p<0.05) as a result of the film condition. For each parameter and for each film condition, means followed by low-case letters (a-c) indicate significant differences as a result of the refrigeration time. No letters are included when no differences (p>0.05) are found.

** Abbreviations of film conditions as expressed in Figure 1.

TABLE 3

Peroxide value and fluorescence ratio assessment* in refrigerated megrim wrapped up with different film conditions**

Quality index	Film condition	Refrigeration storage time (days)			
		0	4	7	11
Peroxide Value (meq. active oxygen kg ⁻¹ lipids)	C	0.18 a (0.01)	6.48 b (0.03)	6.70 c (0.12)	7.23 d (0.39)
	S-0.5	0.18 a (0.01)	6.51 b (0.12)	6.62 b (0.19)	7.17 c (0.16)
	S-1	0.18 a (0.01)	6.55 b (0.12)	6.68 b (0.12)	7.13 c (0.08)
Fluorescence Ratio	C	2.99 (0.87)	3.04 (0.86)	4.03 B (0.56)	3.79 (0.31)
	S-0.5	2.99 (0.87)	2.76 (0.59)	3.13 A (0.05)	3.40 (0.47)
	S-1	2.99 (0.87)	2.80 (0.48)	2.85 A (0.50)	3.22 (0.06)

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

For each parameter and for each refrigeration time, mean values followed by different capital letters (A, B) indicate significant differences ($p < 0.05$) as a result of the film condition. For each parameter and for each film condition, means followed by low-case letters (a-d) indicate significant differences as a result of the refrigeration time. No letters are included when no differences ($p > 0.05$) are found.

** Abbreviations of film conditions as expressed in Figure 1.

TABLE 4

Evaluation of sensory acceptance* in refrigerated megrim wrapped up with different film conditions**

Descriptor	Refrigeration time (days)	Film condition		
		C	S-0.5	S-1.0
Skin and mucus development	0	E	E	E
	4	A	A	A
	7	A	A	A
	11	B	B	B
Eyes	0	E	E	E
	4	A	A	A
	7	A	A	A
	11	B	B	B
External odour	0	E	E	E
	4	A	A	A
	7	B ^b	B ^b	A ^a
	11	C ^b	B ^a	B ^a
Gill appearance and odour	0	E	E	E
	4	A	A	A
	7	B	B	B
	11	C ^b	B ^a	B ^a
Consistency	0	E	E	E
	4	A	A	A
	7	A	A	A
	11	B	B	B
Flesh odour (raw state)	0	E	E	E
	4	A	A	A
	7	B ^b	B ^b	A ^a
	11	B	B	B
Flesh odour (cooked state)	0	E	E	E
	4	A	A	A
	7	B	B	B
	11	C ^b	C ^b	B ^a
Flesh taste	0	E	E	E
	4	A	A	A
	7	B	B	B
	11	C ^b	C ^b	B ^a

* Quality categories: E (excellent), A (good), B (fair) and C (unacceptable). For each chilling time and descriptor, scores followed by different superscripts (a, b) indicate significant ($p < 0.05$) differences as a result of the film condition.

** Abbreviations of film conditions as expressed in Figure 1.

Microbial group	Film condition	Chilling time (days)		
		4	7	11
Aerobes	C	3.66 (1.16)	5.59 (1.77)	6.61 (2.16)
	S-0.5	3.61 (0.99)	5.32 (1.80)	6.17 (1.92)
	S-1	3.43 (0.77)	4.77 (1.47)	6.62 (2.15)
Enterobacteriaceae	C	3.75 (1.15)	3.90 (0.95)	4.30 (1.10)
	S-0.5	3.58 (0.96)	3.81 (0.99)	4.10 (1.10)
	S-1	3.01 (0.94)	2.60 (0.81)	3.95 (0.81)

Figure 1:

Eje Y: Aerobe counts (log CFU g⁻¹ muscle)
Eje X: Chilling time (days)

Figure 2:

Eje Y: Enterobacteriaceae counts (log CFU g⁻¹ muscle)
Eje X: Chilling time (days)

En ambas en la leyenda, simplemente: C, S-0.5 y S-1.

En la cabeza de cada figura pon Figure 1 (ó 2) y los datos entre paréntesis como barras.

Dos documentos independientes, y en eps ó tif (resolución mínima 300 dpi)

FONDO

Rohani-Ghadikolaei, K., Abdulalian, E. & Ng, W. (2011). Evaluation of the proximate, fatty acid and mineral composition of representative green, brown and red seaweeds from the Persian Gulf of Iran as potential food and feed resources. *Journal of Food Science and Technology*, **49**, 774-780.

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