**Preservative effect of algae extracts on lipid composition and
rancidity development in brine-canned Atlantic Chub
mackerel (*Scomber colias*)**

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ABSTRACT

This research focuses on the lipid fraction and the rancidity stability of commercial canned Atlantic Chub mackerel (*Scomber colias*). For it, two different concentrations of aqueous extracts of two abundant algae (*Fucus spiralis* and *Ulva lactuca*) were included in the brine-packing (aq. 2% NaCl) medium during mackerel canning. Quality analyses were carried out after a 3-month canned storage (20°C). Phospholipids, sterols and α -tocopherol contents showed a marked loss as a result of canning, this loss being mostly inhibited when considering the canned fish packed in the presence of the most concentrated algae extracts. Breakdown of free fatty acids and peroxides produced during the thermal treatment was inhibited by the presence of the algae extracts, a higher retention of such molecules being observed by increasing the algae extract concentration. Analysis of fluorescent compounds formation (tertiary lipid oxidation) showed a marked increase after canning, this increase being partly inhibited by the presence of antioxidant compounds present in the algae extracts; interestingly, oxidation inhibition was found more important in canned fish corresponding to *F. spiralis* batches than *U. lactuca*. A preservative effect on lipid constituents and rancidity development is concluded by the presence of algae extracts in the packing medium.

PRACTICAL APPLICATIONS

The extensive heat treatment involved during fish canning has been reported to produce breakdown of constituents, lipid damage (oxidation and hydrolysis) development showing a marked effect on nutritional and sensory properties; furthermore, this effect has shown to be greater when a brine medium is applied as packing system. Marine algae have been described as being a relevant source of chemical constituents including an antioxidant and antibacterial behaviour. In the

current work, employment of a commercial brine-packing including aqueous algae extracts has shown a preservative effect on the lipid fraction (composition and rancidity stability) during the canning process of Atlantic Chub mackerel. The proposed strategy may open the way to the development of a natural biopreservation for commercial canned fish due to the simple methodology employed and the abundance of the two algae chosen for the study. Further research should envisage optimisation of the preservative compounds extraction by advanced technologies.

Key words: Fish canning; brine packing; algae extracts; lipid changes; rancidity stability

Running Head: Canned mackerel lipids and algae extracts

1. INTRODUCTION

Seafood lipids are known to provide high contents of important components for the human diet, such as nutritional lipid-soluble vitamins and essential polyunsaturated fatty acids (PUFA) that have shown a positive role in preventing certain human diseases, including cardiovascular ones [1]. However, due to the high content on long-chain PUFA, marine lipids are highly susceptible to oxidation, this leading to important drawbacks for their commercialisation [2].

Among traditional technologies, canning represents one of the most important means of fish preservation, which can last for several years. Many marine species produce excellent canned products, supporting an important role in human nutrition and commercial value. However, the extensive heat treatment involved substantially alters the nature of the raw material so that a product with different characteristics is obtained [3, 4]. Since both enzymes and bacteria should be permanently inactivated, the main concern reported during fish canning would be breakdown of chemical constituents; in this context, lipid damage (oxidation and hydrolysis) development has proved a marked negative effect on the nutritional and sensory values in the final product.

Previous research has dealt with the effect of the packing medium on the lipid oxidation in canned fish. Thus, a brine medium showed to give rise to a greater development in canned tuna (*Thunnus alalunga*) when compared to different kinds of vegetable oils (namely, refined soybean oil, refined olive oil, and extra-virgin olive oil) [5]; indeed, the high content of phenolic compounds in extra-virgin olive oil showed a marked inhibition of lipid oxidation progress in canned tuna. A greater development of lipid oxidation was also observed by Naseri and Rezaei [6] in brine-canned sprat (*Clupeonella cultriventris*) when compared to its counterpart packed in sunflower oil. Contrary, no effect of the packing medium was inferred for the lipid hydrolysis

development when comparing brine and soybean oil conditions in canned tuna (*T. alalunga*) [7].

Marine algae are reported to include a wide variety of beneficial nutrients [8, 9] and chemical constituents with potential antioxidant and antibacterial activity [10, 11]. However, employment of algae extracts for the quality enhancement of canned fish can be considered scarce. Thus, Ortiz *et al.* [12] included aqueous extracts of various algae (*Durvillaea antarctica*, *Ulva lactuca*, *Pyropia columbina*, *Macrocystis pyrifera* and *Gracilaria chilensis*) during Atlantic salmon (*Salmo salar*) canning carried out by employing a water-packing medium; as a result, a remarkable rancidity stabilisation could be observed throughout an accelerated canned storage condition (up to 140 days at 40 °C). Recently, the addition of an aqueous extract of *Bifurcaria bifurcata* in the water medium employed for packing in canned Atlantic mackerel (*Scomber scombrus*) led to a protective effect on lipid rancidity development [13].

The present research focuses on the lipid fraction (composition and rancidity stability) in canned fish including a commercial brine-packing (aq. 2% NaCl) medium. For it, aqueous extracts of two abundant algae (*Fucus spiralis* and *Ulva lactuca*) were included in the packing medium during Atlantic Chub mackerel (*Scomber colias*) canning. Analyses (lipid content and classes, lipid oxidation and hydrolysis) were carried out in canned fish after a 3-month storage at room temperature. Complementary assessment of volatile amine compounds was carried out.

2. MATERIAL AND METHODS

2.1. Starting algae: Polyphenol content and aqueous extracts

Lyophilised algae *F. spiralis* and *U. lactuca* were provided by Porto-Muiños (Cereda, A Coruña, Spain).

Total polyphenols content of lyophilised algae was assessed by means of the Folin-Ciocalteu colorimetric method (Cary 3E UV–Visible spectrophotometer, Varian; Mulgrave, Victoria, Australia) as described previously [14]. Measurements were made in triplicate, gallic acid being used as standard. Results were calculated as mg gallic acid equivalents (GAE)·g⁻¹ lyophilised alga.

For the preparation of algae extracts, 14 g of each alga were mixed with distilled water (140 mL), stirred for 30 s, sonicated for 30 s and centrifuged at 3000 x g for 10 min at 4 °C. Then, the supernatant was recovered and the process was repeated. Finally, supernatants were pooled together for each alga and made up to 250 mL with distilled water.

2.2. Raw fish and canning process

Specimens (48 fish) of Atlantic Chub mackerel (*S. colias*) (length range: 20-33 cm; weight range: 155-185 g) were obtained at Vigo harbour (North-Western Spain) in May 2018 and transported on ice to the laboratory. Once at the laboratory, 8 individual fish specimens were selected and divided into four groups (two specimens per group). Such specimens were beheaded, eviscerated and filleted, and the white muscle was then analysed within each group independently ($n = 4$), being labelled as R-0 batch (starting raw mackerel; raw control).

Fillets from the remaining fish specimens were obtained as described for the raw fish. Then, 45-g portions of mackerel fillets were placed in small flat rectangular cans (105 × 60 × 25 mm; 150 mL). As packing medium, 10 and 30 mL of each alga extract (corresponding to 0.56 and 1.68 g of extracted alga, respectively) were added to the cans, followed by the addition of 30 mL and 10 mL of distilled water, respectively. Finally, 40 mL of brine solution (4% w/v) were added to all cans. As a result, low-

concentrated (FS-1 batch, *F. spiralis*; UL-1 batch, *U. lactuca*) and high-concentrated (FS-2 batch, *F. spiralis*; UL-2 batch, *U. lactuca*) packing conditions were prepared for each alga, respectively. Canned control samples were prepared by introducing in the can 45 g of fish fillet and adding 40 mL of distilled water and 40 mL of the brine solution (C-O packing condition; control canned batch). For each packing condition, four different cans were prepared that were analysed separately ($n = 4$).

All cans were vacuum-sealed in a horizontal steam retort (115 °C, 45 min; $F_0 = 7$ min) (CIFP Coroso, Ribeira, A Coruña, Spain). Once the heating time was completed, steam was cut off, and air was used to flush away the remaining steam. The cans were cooled at reduced pressure. After 3 months of storage at room temperature (20 °C), the cans were opened, and the liquid part was carefully drained off gravimetrically and filtered through a filter paper. Mackerel white muscle was separated, wrapped in filter paper and used for analysis.

Selection of the alga extract contents in this study was based on several preliminary tests. For the canning conditions, a 1.68-g extract of both algae corresponds to the highest concentration possible, without modifying the sensory descriptors of canned mackerel (i.e., flesh colour, odour and flavour). In order to analyse the effect of the alga content, a lower concentration (namely, 0.56-g extract) was also checked.

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

2.3. Lipid content and lipid classes analysis

Lipids were extracted from the fish white muscle by the Bligh and Dyer [15] method, based on a single-phase solubilisation of the lipids with a chloroform-methanol (1:1) mixture. The results were calculated as g lipid·kg⁻¹ muscle.

Phospholipids (PL) were quantified by measuring the organic phosphorus in the total lipid extracts according to the Raheja *et al.* [16] method, which is based on a complex formation with ammonium molybdate and spectrophotometric (Beckman Coulter, DU 640; London, UK) assessment. Results were calculated as g PL·kg⁻¹ lipids.

Sterols (ST) were determined on total lipid extracts by the method of Huang *et al.* [17] based on the Liebermann-Buchardt reaction. Results were calculated as g ST·kg⁻¹ lipids.

Free fatty acid (FFA) content was determined in the lipid extract of the fish muscle by the Lowry and Tinsley [18] method, which relies on complex formation with cupric acetate-pyridine, followed by spectrophotometric (715 nm) assessment. Results were calculated as g FFA·kg⁻¹ lipids.

The presence of different tocopherol compounds (α , β , γ and δ) was checked. For it, HPLC analysis according to the method of Cabrini *et al.* [19] was applied to the fish white muscle. As a result, only α -tocopherol was detected, its content being calculated with a calibration curve prepared from commercial α -tocopherol. Results are expressed as g α -tocopherol·kg⁻¹ lipids.

2.4. Lipid oxidation assessment

Peroxide value (PV) was determined spectrophotometrically on the lipid extract by peroxide reduction with ferric thiocyanate, according to Chapman and McKay [20]. The results were calculated as meq. active oxygen·kg⁻¹ lipids.

Thiobarbituric acid index (TBA-i) was determined according to Vyncke [21]. This method is based on the reaction between a trichloroacetic acid extract of the fish muscle and thiobarbituric acid. Content of thiobarbituric acid reactive substances (TBARS) was spectrophotometrically measured at 532 nm and calculated from a

standard curve using 1,1,3,3-tetraethoxy-propane (TEP). Results were calculated as mg malondialdehyde·kg⁻¹ muscle.

Fluorescent compounds formation (Fluorimeter LS 45; Perkin Elmer España; Tres Cantos, Madrid, Spain) was measured in the lipid extract. As described previously [22], fluorescence was measured at excitation/emission of 393/463 and 327/415 nm. The relative fluorescence (RF) was calculated as follows: $RF = F/F_{st}$, where F is the fluorescence measured at each excitation/emission wavelength pair and F_{st} is the fluorescence intensity of a quinine sulphate solution (1 µg·mL⁻¹ in 0.05 M H₂SO₄) at the corresponding wavelength pair. The fluorescence ratio (FR) was calculated as the ratio between the two RF values: $FR = RF_{393/463\text{ nm}}/RF_{327/415\text{ nm}}$.

Lipid extracts were converted into fatty acid methyl esters (FAME) by using acetyl chloride and then analysed by gas-liquid chromatography (Perkin-Elmer 8700 chromatograph; Madrid, Spain), according to Aubourg *et al.* [23]. Peaks corresponding to FAME were identified by comparing their retention times with those of standard mixtures (Qualmix Fish, Larodan, Malmo, Sweden; FAME mix, Supelco, Inc., Bellefonte, PA, USA). Peak areas were automatically integrated, C19:0 fatty acid being used as the internal standard for quantitative purposes. The polyene index (PI) was calculated as the following fatty acids contents ratio: $(C20:5\omega3 + C22:6\omega3)/C16:0$.

2.5. Volatile amines formation

Total volatile base-nitrogen (TVB-N) values were measured as reported by Antonacopoulos [24], with some modifications. Briefly, fish muscle (10 g) was extracted with 60 g·L⁻¹ perchloric acid in water (30 mL) and brought up to 50 mL. An aliquot of the acid extracts was rendered alkaline to pH 13 with 200 g·L⁻¹ aqueous

NaOH and then steam-distilled. Finally, the TVB-N content was determined by titration of the distillate with 10 mM HCl. Results are expressed as mg TVB-N·kg⁻¹ muscle.

Trimethylamine-nitrogen (TMA-N) values were determined using the picrate colorimetric method, as previously described by Tozawa *et al.* [25]. This method involved the preparation of a 5% trichloroacetic acid extract of fish muscle (10 g/25 mL). Results were calculated as mg TMA-N·kg⁻¹ muscle.

2.6. Statistical analysis

All data obtained were evaluated by analysis of variance (ANOVA) to explore differences resulting from the presence of the algae extracts in the packing medium. Average values were compared using the least-squares difference (LSD) method. Differences among batches were considered significant for a confidence interval at the 95% level ($p < 0.05$) in all instances. PASW Statistics 18 software for Windows (SPSS Inc., Chicago, IL, USA) was used throughout.

3. RESULTS AND DISCUSSION

3.1. Lipid content and lipid classes analysis

Lipid content (Table 1) of the starting fish can be considered as relatively low (13.66 g kg⁻¹ muscle) taking into account that a fatty species is encountered. This value can be explained on the basis that fish specimens were obtained in spring time, a period when the lipid content is reported to be lower than in other seasons [26, 27]. A marked lipid content increase ($p < 0.05$) was observed as a result of the canning process (comparison between R-0 and C-0 batches). This increase can be explained as a consequence of a marked water loss of fish muscle during the sterilisation process [22,

28]. No significant effect ($p > 0.05$) on lipid content was inferred by the presence of both algae extracts in the packing medium.

In agreement with the well-known inverse relationship between total lipid and PL contents, PL levels obtained can be considered as relatively high in all kinds of samples (Table 1) [26, 29]. This general relationship has been explained on the basis that PL are reported to be a lipid group with structural functions, not being affected by the lipid accumulation resulting from feeding. A marked loss ($p < 0.05$) of PL could be observed as a result of the canning process (comparison between R-0 and C-0 batches). This loss could be explained as a result of two mechanisms. One side, as supporting a high PUFA content [23], PL could be damaged easily by the heating process. On the other, as being a group including relatively polar lipid classes, PL could be leached partly into the packing medium and be lost from the muscle. Comparison among canned samples showed scarce significant differences. However, average values revealed a higher PL presence in samples being packed under conditions including algae extracts, this effect being stronger by increasing the alga content. Consequently, a partial protective effect during heating and/or an inhibitory effect on PL leaching to the packing medium can be inferred. Interestingly, a significantly higher ($p < 0.05$) retention of PL compounds was observed in fish corresponding to the FS-2 packing condition when compared to both controls. Furthermore, *F. spiralis* packaging led to higher average values than *U. lactuca*.

As in the case of PL, ST compounds are considered a functional class with structural roles in living bodies and not being affected especially by lipid accumulation [26, 29]. In the current study, sterols content showed scarce significant differences among samples under study (Table 1). A decrease of the average content could be observed as a result of canning (comparison between R-0 and C-0 batches). As for PL,

this content decrease could be explained on the basis of being damaged during the thermal processing and be lost into the packing medium. However, analysis of average values showed that this general decrease was partly inhibited by the presence of algae extracts in the packing medium when taking into account samples corresponding to the highest concentrations (i.e., FS-2 and UL-2 batches). Indeed, a significantly higher ($p < 0.05$) value was obtained for canned fish belonging to the UL-2 batch when compared to the canned control.

The content of α -tocopherol did not provide differences ($p > 0.05$) as a result of the canning process (Table 1); neither by the presence of the alga extracts in the packing medium. However, taking into account the average values, a decrease was inferred after the canning process (comparison between R-0 and C-0 batches); additionally, fish canned under packing conditions including the highest concentrations of both algae extracts led to higher average values than their corresponding fresh and canned controls. Results could be explained on the basis that α -tocopherol would be partially lost during the thermal treatment in agreement to its protective role for avoiding rancidity development in fish muscle; however, the presence in the packing medium of antioxidant compounds included in the algae extracts would provide a protective effect on α -tocopherol [30, 31]. Comparison between both algae in the current study showed higher average values at both concentrations in fish belonging to the *F. spiralis*-packing systems. Consequently, a partially higher effect on the vitamin E retention can be inferred for *F. spiralis* extracts.

An inhibitory effect on α -tocopherol loss was also observed by Ortiz *et al.* [12] by including an *U. lactuca* extract in the water-packing medium of canned salmon (*S. salar*) stored at 40 °C for a 170-day storage. Furthermore, a previous refrigerated storage under slurry ice condition led to a higher retention of α -tocopherol in canned

sardine (*Sardina pilchardus*) when compared to fish that had been previously stored under traditional ice [28]. A preservative effect has also been described in frozen (–10 °C) minced fish (mackerel, *Scomber scombrus*; horse mackerel, *Trachurus trachurus*) when a previous treatment with a polyphenolic extract obtained from grape by-products was applied [30]. A similar effect on the α -tocopherol content retention was observed in refrigerated (4 °C) minced horse mackerel (*T. trachurus*) in the presence of a grape procyanidins extract [31].

FFA contents are expressed in Fig. 1. This lipid class has been employed as a valuable measurement for the lipid hydrolysis development in most seafood. Concerning its formation during a fish canning process, two opposite effects are likely to occur [4, 32]. One side, the heating process can produce the hydrolysis development of high-molecular-weight lipid classes like PL and triacylglycerides (TG). On the other, as being low-molecular-weight molecules, FFA are likely to be fastly oxidised or broken down by the heating process as providing a greater accessibility to oxygen and other pro-oxidant molecules when compared to TG and PL. Thus, an average value increase was observed in the present work as a result of the canning process (comparison between R-0 and C-0 controls) in agreement to previous related research [22, 32]. Interestingly, the presence of algae extracts in the packing medium showed an inhibitory effect on the development of the second factor, so that higher FFA values were present in all alga-canned samples when compared to control canned fish. This higher retention was found significant ($p < 0.05$) in the case of the most concentrated packing condition corresponding to *F. spiralis*. Consequently, a protective effect on FFA breakdown could be inferred. A preservative effect on FFA content was also observed by Barbosa *et al.* [13] when including *B. bifurcata* extracts in the water-packing medium of canned Atlantic mackerel (*S. scombrus*).

3.2. Lipid oxidation determination

A low peroxide formation could be observed in all kinds of samples (< 8.0 ; Table 2) in agreement to previous research related to other canned species such as Atlantic bluefin tuna (*Thunnus thynnus*) and sardine (*Sardina pilchardus*) [33] and Atlantic salmon (*Salmo salar*) [12]. In the current study, peroxide assessment showed a marked decrease ($p < 0.05$) as a result of the canning process (comparison between raw and canned control samples) (Table 2). However, canned samples including an algae extract in the packing medium showed higher ($p < 0.05$) levels than the canned control. During the sterilisation step, peroxides are expected to be produced as a result of the thermal treatment; however, this treatment is also reported to destroy primary oxidation compounds and lead to low-molecular-weight molecules formation [22, 28]. It can be concluded that both algae extracts have avoided partly the peroxide destruction during the thermal treatment, this effect being higher ($p < 0.05$) by increasing the algae extract presence. Interestingly, both algae showed a similar protective effect on peroxide compounds.

The assessment of secondary oxidation compounds (i.e., TBA index) did not provide ($p > 0.05$) any effect as a result of the canning process or the presence of the algae extracts in the packing medium (Table 2). As an explanation for this result, compounds measured by this quality index are expected to be produced during the thermal treatment (namely, peroxides breakdown), but would be lost partly into the brine packing medium and not measured when analysing the fish muscle. Consequently, their determination would not afford an accurate measurement of quality loss. A marked decrease of the TBA-i has already been described for canned fish related to water- [13], tomato-sauce- [33] and oil-packing [5] conditions.

Determination of tertiary lipid oxidation showed a significant increase ($p < 0.05$) after the canning process (comparison between fresh and canned control samples) (Fig. 2). Similarly, previous studies have proved that canning process would lead to a marked formation of fluorescent compounds, namely, sardine (*Sardina pilchardus*) [22], Coho salmon [32] and sprat [6]. In the current study, the presence of all algae extracts in the packing medium led to lower average values when compared to the C-0 batch. Interestingly, differences were found significant ($p < 0.05$) when considering both kinds of samples including *F. spiralis* extracts (i.e., FS-1 and FS-2 batches). Consequently, an inhibitory effect on lipid oxidation development is inferred for this alga extract. In agreement to the current research, Barbosa *et al.* [13] showed an inhibitory effect on the FR value in canned Atlantic mackerel (*S. scombrus*) by including *B. bifurcata* extracts in the water-packing medium. A lower FR value was also observed in canned sardine (*S. pilchardus*) that was previously stored under slurry ice conditions when compared to traditional ice [28].

The polyene index (Table 2) did not reveal significant differences ($p > 0.05$) among the different samples according to previous research on canned seafood [6]. However, average values indicated a partial loss of PUFA compounds after the canning process, this loss being avoided by the presence of both algae extracts in the packing medium. Thus, for both algae, higher average values were obtained by increasing the alga extract content in the packing medium, a 1.97 score being attained in samples corresponding to the highest concentration of both algae. In a closely related work, Ortiz *et al.* [12] showed a significant PI retention in canned Atlantic salmon (*S. salar*) muscle when packed in a water medium including an ulte (basal part of *D. antarctica*) extract; however, no differences were obtained in such study when other algae

(cochayuyo, frond of *D. antarctica*; *U. lactuca*; *P. columbina*) extracts were included in the water-packing systems.

In agreement to the different lipid oxidation indices determined in the current experiment, a partial inhibition of the development of this damage pathway has been attained by the presence of both algae extracts in the brine-packed medium. Interestingly, this effect was found more important in the case of *F. spiralis* use. In agreement with this difference, a valuable polyphenol content (53.5 ± 5.0 mg GAE·g⁻¹ lyophilised alga) was measured in *F. spiralis* samples employed in the current study, while *U. lactuca* showed a markedly lower polyphenol content (0.7 ± 0.6 mg GAE·g⁻¹ lyophilised alga). However, previous research has reported on the antioxidant behaviour of *U. lactuca*, this activity being explained by the presence of preservative molecules such as tocopherols [8], carotenoids [34], phenolic compounds [35] and sulphates [36].

Concerning *F. spiralis* extracts, the antioxidant capacity and reducing ability of this alga has already been proved by means of *in-vitro* DPPH and FRAP tests [11]. Thus, a marked content on polyphenols [10] and α -tocopherol [9] has been reported, as well as the presence of phlorotannins [37]. Furthermore, a comparative study on different kinds of extracts showed that a combined extract of ethanol and water would lead to a greater antioxidant effect in chilled fish than ethanol or water extracts alone [38]. In general, extraction with alcoholic solvents has been reported to provide higher phenol compound contents [39, 40], although water extraction would lead to higher yields [10, 41]. Thus, hydrophilic-type molecules, such as proteins, peptides and polysaccharides, would be extracted and lead to marked preservative effects [10, 42, 43] in agreement to the current study. In order to optimise the extraction of bioactive compounds supporting an antioxidant behaviour, further research focussed on the

application of advanced extracting technologies such as high-pressure, CO₂-supercritic or ultrasound conditions is expected to be developed [44, 45].

3.3. Assessment of volatile amines content

Values on TVB-N showed a marked decrease ($p < 0.05$) after the canning process (Table 3). In agreement to previous related research [28, 46], two opposite factors can be outlined affecting their content. One side, the heating process leads to the formation of volatile compounds (e.g., ammonia, methylamine, dimethylamine, trimethylamine) by breakdown of different kinds of constituents present in the fish muscle. On the other, since most of such volatile amines produced are water-soluble, they would leach into the packing medium, be lost from the muscle, and consequently, not measured in the current index. Results show that the second effect has been especially important, so that all canned samples depicted lower ($p < 0.05$) values than the raw fish. Furthermore, comparison among canned samples showed scarce significant differences, so that a general trend could not be implied about the effect of the presence of algae extracts in the packing medium.

In agreement to the present results, TVB-N levels did not provide differences in canned Atlantic mackerel (*Scomber scombrus*) that was water-packed under different concentrations of alga *B. bifurcata* [13]. Opposite results were obtained when an oil-packing medium was concerned. Thus, TVB-N content showed to increase after the canning process in canned salmon (*O. kisutch*) [32], tuna (*Thunnus thynnus*) and sardine (*S. pilchardus*) [33], and sardine (*S. pilchardus*) [28].

Concerning the TMA content, a sharp increase ($p < 0.05$) is observed as a result of canning (Table 3) in agreement to previous seafood canning studies including an oil-packing media [32, 46]. Differently to most volatile amines, TMA is a non-water-

soluble tertiary amine so that it is not likely to be leached into the present brine packing medium. According to the current work, a marked TMA content increase was observed after canning in tuna (*T. thynnus*) and sardine (*S. pilchardus*) muscle [33]. Comparison among canned samples did not provide significant differences ($p > 0.05$), so that a general trend about algae extract presence on the formation of this deteriorative molecule could not be implied. In agreement to the present research, no differences on TMA content could be observed by Barbosa *et al.* [13] when comparing canned mackerel (*S. scombrus*) samples including a water-packing medium with different quantities of alga *B. bifurcata* extract.

4. CONCLUSIONS

In the current work, employment of a commercial brine-packing (aq. 2% NaCl) including aqueous algae extracts has shown a preservative effect on the lipid fraction (composition and rancidity stability) during the canning process of Atlantic Chub mackerel. Thus, PL, ST and α -tocopherol contents showed a marked loss as a result of canning, this loss being mostly inhibited when considering the canned fish packed in the presence of the most concentrated algae extracts. Breakdown of FFA and peroxides produced during the thermal treatment was inhibited by the presence of the algae extracts, a higher retention of such molecules being observed by increasing the algae extract concentration. Analysis of fluorescent compounds formation (tertiary lipid oxidation) showed a marked increase after canning, this increase being partly inhibited by the presence of antioxidant compounds present in the algae extracts; interestingly, oxidation inhibition was found more important in canned fish corresponding to *F. spiralis* batches. Finally, the algae extracts presence led to negligible differences for the

thiobarbituric acid and polyene indices, as well as for the volatile amines (total and TMA) formation.

The proposed strategy may open the way to the development of a natural biopreservation alternative for commercial canned fish due to the simple methodology employed and the abundancy of the two algae chosen for the study. Further research is envisaged to optimise the preservative compounds extraction by advanced methodologies.

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CONFLICT OF INTEREST

The authors have declared no conflict of interest.

FIGURE LEGENDS

Figure 1: Free fatty acid (FFA) content ($\text{g}\cdot\text{kg}^{-1}$ lipids)* in fresh and canned mackerel muscle including different packing systems**

* Average values of four replicates ($n = 4$); standard deviations are indicated by bars. Values accompanied by different letters (a, b, c) indicate significant ($p < 0.05$) differences.

** Abbreviations employed for fresh and canned samples as expressed in Table 1.

Figure 2: Assessment of the fluorescence ratio* in fresh and canned mackerel muscle including different packing systems**

* Average values of four replicates ($n = 4$); standard deviations are indicated by bars. Values accompanied by different letters (a, b, c) indicate significant ($p < 0.05$) differences.

** Abbreviations employed for fresh and canned samples as expressed in Table 1.

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Fig. 1

Fig. 2