Impact of a packing medium with alga *Bifurcaria bifurcata* extract on canned Atlantic mackerel (*Scomber scombrus*) quality

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ABSTRACT

BACKGROUND: The present research focused on the quality of canned fish. Its primary objective was the quality enhancement of canned Atlantic mackerel (*Scomber scombrus*) by including an aqueous *Bifurcaria bifurcata* extract in the packing medium. Various alga extract concentrations were tested and compared to a control without alga extract. After a 3-month canned storage, the cans were opened, and quality changes in fish white muscle were analyzed.

RESULTS: An inhibitory effect on the lipid oxidation development (tertiary compounds formation) and on color parameters (L* and b*) values was observed as a result of the alga presence in the packing medium. On the contrary, the presence of the alga extract did not produce any effect on the formation of volatile compounds (total and trimethylamine) and the lipid hydrolysis (free fatty acids formation) development.

CONCLUSION: A preservative effect derived from the use of an aqueous *B. bifurcata* extract as packing medium is concluded, and this result is primarily linked to the presence of hydrophilic preservative molecules. The packing system proposed in this work constitutes a novel and promising strategy to enhance the quality of commercial canned fish products.

Running Title: Canned mackerel quality and *Bifurcaria bifurcata*

Key Words: *Bifurcaria bifurcata*; aqueous extract; mackerel; canning; packing medium; lipid oxidation

INTRODUCTION

Many marine species are suitable for canning, which guarantees excellent nutritional standards with and great economical values. As a result of the thermal process involved, endogenous enzymes and bacteria should be inactivated provided reinfection does not occur and no negative interaction with the container is produced.[1,2] However, most constituents of marine species are known to be particularly labile to heat treatment, so that important degradative events may occur, causing marked nutritional quality losses.[3,4] Among chemical constituents, marine lipids are known to possess a high content of polyunsaturated fatty acids. During heat treatment, polyunsaturated fatty acids can be oxidized, leading to browning, flavor changes and loss of essential nutrients. Consequently, previous research has shown canned fish quality depends strongly on several factors, such as previous storage conditions,[5] coating medium[6] or time-temperature sterilization conditions.[7]

Algae are exposed to a combination of high oxygen concentration and light. The lack of structural damage in their organs has led to consider that their protection against oxidation arises from their content on antioxidant substances.[8] Consequently, marine algae are receiving increasing attention as a source of bioactive compounds (e.g., polyphenols, alkaloids, terpenes, phycocyannins, carotenoids) with antioxidant activity.[9-11] Furthermore, these species are also recognized as an important source of beneficial nutrients, such as lipids, amino acids, vitamins, trace minerals, and dietary fibers.[12,13]

Bifurcaria bifurcata has shown to be abundantly present in the South-West coasts of Ireland and England, in the Atlantic coasts of France and Spain, as well as in the Portugal coasts.[14,15] The proximate composition of this brown macroalga has been described.[16] Furthermore, isolation and identification of various kinds of compounds in

its composition, such as phenols,[17] diterpenes,[18] sterols[19] and polysaccharides[20] has been carried out. Additionally, the antioxidant activity of this alga has been reported *in vitro*[10] and on chilled fish studies.[21]

As being the most relevant event during canning and canned storage, the present research focused on the effect of the sterilization step on the quality of canned fish. Thus, its primary objective was the quality enhancement of canned Atlantic mackerel (*Scomber scombrus*) by including an aqueous *B. bifurcata* extract in the packing medium. For it, quality changes were analyzed after a 3-month canned storage by evaluating lipid damage development (oxidation and hydrolysis), formation of volatile amines (total and trimethylamine) and muscle color changes. This storage time was selected as being convenient to analyze the effect on the canned product quality of the alga presence in the coating medium during the sterilization step.

MATERIALS AND METHODS

Preparation of *B. bifurcata* extracts, initial raw fish and canning

Lyophilized alga *B. bifurcata* (12.5 g) (Porto-Muiños, Cerceda, A Coruña, Spain) was mixed with 140 mL distilled water, stirred for 30 s, sonicated for 30 s and centrifuged at 3,500*xg* at 4 ºC for 10 min. Then, the supernatant was recovered, and the extraction process was repeated three more times. Finally, all supernatants were pooled together and completed to 500 mL with distilled water.

Specimens (60 fish) of Atlantic mackerel (*S. scombrus*) (weight range: 245–290 g; length range: 30–34 cm) were obtained at Vigo harbor (North-Western Spain) and transported on ice to the laboratory. Upon arrival at the laboratory, 10 individual fish specimens were selected and divided into five groups (two specimens per group). These

raw fish specimens were beheaded, eviscerated and filleted, and the white muscle was then analyzed independently (n=5).

The remaining fish specimens were processed as described above for the raw. Consequently, 40 g portions of mackerel muscle were placed in small flat rectangular cans (105 × 60 × 25 mm; 150 mL). For the packing medium, 5, 10, 25 and 50 mL of the alga extract (corresponding to 0.125, 0.250, 0.625 and 1.250 g of extracted alga, respectively) were added to the cans, labeled as PS-1, PS-2, PS-3 and PS-4 conditions, respectively. Then, the cans were filled with distilled water. The control was prepared with only distilled water as packing medium (PS-0 condition). For each packing condition, five different cans were prepared that were analyzed separately (n=5).

The cans were vacuum-sealed in a horizontal steam retort (115 ºC, 45 min; F_o = 7 min) (Justo López Valcárcel S. A., Vigo, 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 (18–20 ºC), 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 tested, a 1.250-g extract of *B. bifurcata* corresponds to the highest concentration possible, without modifying the sensory descriptors of canned mackerel (flesh color, odor and flavor).

Lipid damage assessment

Lipids were extracted from the fish white muscle by the Bligh and Dyer method,[22] based on a single-phase solubilization of the lipids with a chloroform-methanol (1:1) mixture. The results were calculated as g lipid kg^{-1} muscle.

Free fatty acid (FFA) content was determined in the lipid extract of the fish muscle by the Lowry and Tinsley method,[23] which relies on complex formation with cupric acetate-pyridine, followed by spectrophotometric (715 nm) assessment. Results were expressed as g FFA kg^{-1} lipids.

Conjugated diene (*CD*) and triene (*CT*) formation were monitored by measuring the absorption of the lipid extract at 233 and 268 nm, respectively.[24] Results were expressed according to the following formula: CD (or CT) = $B \times V/w$, where B is the absorbance reading at 233 (or 268) nm, V is the volume (mL), and w is the mass (mg) of the lipid aliquot measured.

Peroxide value (PV) was determined spectrophotometrically (Beckman Coulter, DU 640; London, UK) on the lipid extract by peroxide reduction with ferric thiocyanate, according to Chapman and McKay.[25] The results were expressed as meq active oxygen kg^{-1} lipids.

Tertiary lipid oxidation compounds, arising from the interaction between oxidized lipids and nucleophilic compounds (namely, protein-like molecules) were measured by fluorescence spectroscopy (Fluorimeter LS 45; Perkin Elmer España; Tres Cantos, Madrid, Spain). As described previously,[26] fluorescence was measured at excitation/emission of 393/463 and 327/415 nm in the lipid extract of the fish muscle. The relative fluorescence (*RF*) was calculated as follows: $RF = F/Fst$, where F is the fluorescence measured at each excitation/emission wavelength pair and Fst is the fluorescence intensity of a quinine sulfate solution (1 µg mL^{-1} in 0.05 M H_2SO_4) at the

corresponding wavelength pair. The fluorescence ratio (*FR*) was calculated as the ratio between the two *RF* values: $FR = RF_{393/463\ nm}/RF_{327/415\ nm}$.

Lipid extracts were converted into fatty acid methyl esters (FAME) by using acetyl chloride and then analyzed by gas-liquid chromatography (Perkin-Elmer 8700 chromatograph; Madrid, Spain), according to an established procedure.[7] 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.). Peak areas were automatically integrated. C19:0 fatty acid was used as the internal standard for quantitative purposes. The polyene index (PI) was calculated as the following fatty acids contents ratio: (C20:5ω3 + C22:6ω3)/C16:0.

Volatile amines formation

Total volatile base-nitrogen (TVB-N) values were measured as reported by Antonacopoulos,[27] 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 were expressed as mg TVB-N kg^{-1} muscle.

Trimethylamine-nitrogen (TMA-N) values were determined using the picrate colorimetric (Beckman Coulter, DU 640; London, UK) method, as previously described by Tozawa, Erokibara and Amano.[28] This method involved the preparation of a 5% trichloroacetic acid extract of fish muscle (10 g/25 mL). Results were expressed as mg TMA-N kg^{-1} muscle.

Instrumental color analysis

Color parameters (L^*, a^* and b^*) were measured by instrumental color analysis (CIE 1976), performed with a tristimulus Hunter Labscan 2.0/45 colorimeter. For each sample analysis, color scores were averaged over four measurements, taken by rotating the measuring head 90° between triplicate measurements per position.

Statistical analysis

All data obtained were evaluated by analysis of variance (ANOVA), to explore differences resulting from the effect of the presence of the alga extract in the packing medium. The averages 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.

RESULTS AND DISCUSSION

Lipid oxidation assessment

Since a fatty fish species (100-125 g lipids kg^{-1} muscle) and a thermal treatment were encountered, various and complementary quality indices related to lipid oxidation development were taken into account.

Assessment of *CD* formation provided scarce differences among the various samples studied (Table 1). Thus, no significant differences ($p>0.05$) could be observed between the initial raw fish and any of the canned samples, in agreement with previous research on canned yellowfin tuna (*Thunnus albacora*).[7] Nonetheless, an increasing average *CD* value could be observed in canned fish with increased amount of alga extract in the packing medium.

198 *CT* detection (Table 1) led to a general increase as a result of canning and
199 canned storage that was found significant ($p<0.05$) in canned samples corresponding to
200 PS-1, PS-3 and PS-4 conditions. A comparison among the various canned samples
201 showed in most instances an increasing tendency of *CT* formation with the *B. bifurcata*
202 extract content.

203 Concerning the PV assessment (Table 1), the average value increased as a result
204 of canning and canned storage (comparison between raw fish and control canned fish).
205 Furthermore, all canned fish corresponding to packing systems including alga extracts
206 showed higher ($p<0.05$) PV than control canned fish. A comparison among the canned
207 samples showed higher ($p<0.05$) PV levels for fish packed in the media containing the
208 two highest alga contents (i.e., PS-3 and PS-4). However, the PV obtained in all cases
209 remained below the 8.3 score. A low peroxide content has also been detected in canned
210 Atlantic bluefin tuna (*Thunnus thynnus*) and sardine (*Sardina pilchardus*),[29] as well as
211 in canned Atlantic salmon (*Salmo salar*).[30]

212 Formation of the primary oxidation products can be considered to result from
213 two opposite reactions. On the one hand, the thermal treatment oxidizes lipids,
214 producing *CD* and *CT* and peroxides. On the other hand, the heat treatment itself can
215 cause the degradation of such molecules. Consequently, a retention tendency for certain
216 types of molecules (particularly, for peroxides) has been observed in the present study
217 by increasing the alga presence.

218 Complex formation as a result of interaction between oxidized lipids (i.e.,
219 primary and secondary) and nucleophilic molecules (mainly protein-type
220 compounds)[31,32] was measured by formation of fluorescent compounds (i.e., *FR*; tertiary
221 lipid oxidation compounds; Figure 1). As a result of canning and canned storage, a
222 marked *FR* increase ($p<0.05$) was implied (comparison between raw fish and control

canned fish), this revealing an increased interaction compounds formation in agreement with a relevant lipid damage development. This increasing trend in the *FR* value was also noted in previous research on canned sardine (*S. pilchardus*),[26] coho salmon (*Oncorhynchus kisutch*)[33] and sprat (*Clupeonella cultriventris*).[6] A comparison among the various canned samples showed a decreasing tendency of the *FR* with increasing presence of the alga extract in the packing medium. Interestingly, canned fish corresponding to the two most concentrated alga conditions showed lower ($p<0.05$) levels than control canned samples. Accordingly, an inhibitory effect on the formation of tertiary lipid oxidation compounds was achieved by including the alga extract in the packing system. Taking into account the whole lipid oxidation process, it can be concluded that this result agrees with the assessment of primary oxidation compounds (namely, peroxides), mentioned above. Thus, the alga extract presence in the coating medium has favored a lower breakdown of primary compounds (i.e., PS-3 and PS-4 canning conditions), so that a lower formation of tertiary ones was produced. Consequently, an inhibition of the lipid oxidation process can be implied by the presence of the alga extract in the coating medium.

The inhibitory effect of aqueous *B. bifurcata* extracts on the lipid oxidation development (i.e., *FR* value) can be explained by the high level of polyphenol compounds previously detected in this alga (40.8±8.3 gallic acid equivalents g^{-1} lyophilized alga)[21] and of previous related research. For instance, various polyhydroxyphenyl ethers and phenyl ethers have been isolated from this alga.[17] On the basis of various kinds of *in vitro* tests, the antioxidant behavior of aqueous, methanolic and dichloromethane extracts of the alga has been proven.[10,15,34] Recently,[21] an ethanolic extract of this alga was included in the icing medium applied during the chilled storage

247 of megrim (*Lepidorhombus whiffiagonis*), which showed an inhibitory effect on lipid
248 oxidation development and microbial activity in chilled fish muscle.

249 Typically, extraction with alcoholic solvents achieves a high total phenolic
250 content.[35,36] However, and in agreement with the preservative effect found in the present
251 research, water extraction of algae has been reported to provide, in most instances, the
252 highest yields.[37,38] Consequently, the majority of water-soluble (or hydrophilic-type)
253 molecules, such as proteins, peptides and polysaccharides, would be extracted and lead
254 to a preservative effect, as verified in previous studies.[39,40] Thus, closely related to the
255 present research, the inclusion in the packing medium of preservative hydrophilic
256 compounds obtained by aqueous extraction of various algae (*Durvillaea antarctica*,
257 *Ulva lactuca*, *Pyropia columbina*, *Macrocystis pyrifera* and *Gracilaria chilensis*), led to
258 a remarkable rancidity stabilization in canned Atlantic salmon (*S. salar*).[30] However,
259 unlike the present research, the study by Ortiz *et al*.[30] involved an accelerated canned
260 storage condition (up to 140 days at 40 ºC).

261 A lower lipid oxidation development was also obtained in canned albacore
262 (*Thunnus alalunga*), by applying several vegetable oils (extra virgin olive oil, refined
263 olive oil and refined soybean oil, respectively) as packing media, when compared to
264 brine.[41] Particularly, extra virgin olive oil was found to exert the highest preservative
265 efficacy due to its comparatively higher content of phenolic compounds. Thus, the
266 antioxidant ability was attributed to the solubilization of hydrophilic phenols at the
267 water-muscle interface. In a related study, Naseri and Rezaei[6] analyzed the lipid
268 oxidation development in canned sprat (*C. cultriventris*) packed under various
269 conditions and observed a greater development of fluorescent compounds in fish packed
270 in brine compared to its counterpart packed in sunflower oil.

The assessment of the PI in the present research did not provide differences ($p>0.05$) among the samples studied (Table 1). Consequently, no effect on this quality index could be implied as a result of the canning process or the *B. bifurcata* presence in the packing medium employed. Similarly, no effect on the PI of canned sprat (*C. cultriventris*) was observed with brine as the packing medium instead of sunflower oil, for a 3-year canned storage.[6] On the contrary, Ortiz *et al.*[30] obtained a significant PI retention in canned Atlantic salmon (*S. salar*) muscle when packed in an aqueous extract of ulte (basal part of alga *D. antarctica*), while no differences were obtained when other algae (cochayuyo, frond of *D. antarctica*; sea lettuce, *U. lactuca*; red luche, *P. columbina*) were tested as packing systems. Furthermore, a marked effect of the sterilization conditions (time and temperature) on the PI was confirmed by Aubourg *et al.*[7] The study demonstrated that for the same F_o value, the condition that included the highest temperature (130 ºC) but the shortest time (27 min) did not provide differences when compared to the raw material. Conversely, other sterilization conditions (110 ºC for 120 min and 115 ºC for 60 min) led to significant PI decreases in canned samples.[7]

Lipid hydrolysis determination

Higher average FFA values (Table 2) were obtained in all canned samples when compared with the raw fish muscle. Lipid hydrolysis development during heat treatment has already been documented in previous research concerning canned albacore (*T. alalunga*)[7] and coho salmon[33] as a result of hydrolysis of high-molecular weight lipid molecules such as triacylglycerides and phospholipids. However, scarce significant differences could be observed among the various canned samples under study, so that a definite trend about the effect of the presence of the alga extract on the lipid hydrolysis development could not be concluded.

296 This lack of tendency among canned fish for FFA formation can be explained on
297 the basis that two opposing mechanisms would be involved during the canning
298 process.[5,33] On the one hand, FFA formation would be expected to be produced via
299 degradation of large-sized lipid molecules (i.e., triacylglycerides and phospholipids)
300 during the thermal treatment. On the other hand, FFA are known to be oxidized faster
301 than higher molecular weight lipid classes (triacylglycerides and phospholipids) by
302 providing a greater accessibility (lower steric hindrance) to oxygen and other pro-
303 oxidant molecules. In concurrence with the present results, Medina *et al.*[42] showed that
304 the extent and mechanism of lipolysis were not influenced by the packing medium
305 (brine and soybean oil) when considering canned albacore (*T. alalunga*).
306
307 **Volatile amines content**
308 As a result of thermal treatment included in the canning process, volatile amines are
309 known to be produced in canned products by breakdown of various muscle constituents
310 such as proteins and trimethylamine oxide.
311 In the present study, a higher TVB-N value was observed for the starting raw
312 fish compared to any canned product so that a marked decrease ($p<0.05$) in the total
313 volatile amines could be implied as a result of the canning process (Table 2). Among
314 canned samples, scarce significant differences were observed, so that a general trend
315 concerning the alga presence in the coating medium could not be concluded.
316 Previous research has shown a marked increase in TVB-N content as a result of
317 the canning process.[43,44] This quality parameter quantifies a broad range of basic
318 volatile compounds (e.g. ammonia, methylamine, dimethylamine, trimethylamine),
319 most of them highly soluble in aqueous media. Consequently, the aqueous packing
320 medium may act as an extracting system, so that volatile compounds would have

leached into the packing liquid and led to a corresponding content decrease in canned fish muscle.

Despite employing sunflower oil as packing system, no significant differences were noted when comparing the TVB-N contents in canned sardine (*S. pilchardus*) stored under different chilling conditions.[44] In another previous research,[30] TVB-N formation was analyzed in canned salmon (*S. salar*) stored under accelerated conditions (up to 140 days at 40 ºC). Interestingly, an inhibitory effect on TVB-N values was observed at the end of the storage experiment in canned fish packaged in the presence of diverse algae extracts (*U. lactuca* and *P. columbina*).[30]

Concerning the TMA formation (Table 2), a sharp increase ($p<0.05$) in all the canned samples was detected relative to the raw fish. Consistent with its chemical structure (i.e., tertiary amine), TMA is known to be scarcely soluble in aqueous media, so that it is unlikely to leach into the packing medium. Among the canned samples, no significant differences ($p>0.05$) could be observed, so that a definite effect on TMA formation of *B. bifurcata* extract, could not be implied.

Previous research has shown a marked TMA formation as a result of cooking and sterilization processes.[33,43] Such formation was reported to be produced from thermal degradation of trimethylamine oxide. However, an inhibitory effect on TMA-N content in canned sardine (*S. pilchardus*) was implied as a result of previously applying an advanced chilling system (i.e., liquid ice) instead of traditional flake ice.[44]

Assessment of color changes

The *L** value increased rapidly as a result of the canning process (comparison of samples corresponding to raw fish and PS-0 packing condition; Table 3). Previous research has also shown an L* value increase in fish species as a result of heat

346 treatment.[33,45] However, due to the presence of alga extract in the packing medium, an
347 inhibition of the *L** increase could be implied in the current study. This inhibition
348 increased with the alga extract presence in the packing system. Interestingly, no
349 differences ($p>0.05$) occurred between raw fish values and canned mackerel packaged
350 with the highest alga content. It is concluded that an inhibitory effect on the lightness
351 increase was obtained by including *B. bifurcata* extract in the packing medium.

352 The assessment of the *a** color parameter did not provide valuable results (data
353 not shown) so that a definite effect of canning or the alga presence in the packing
354 medium could not be concluded on this color parameter.

355 A comparison between raw fish and canned control samples showed a marked
356 *b** value increase ($p<0.05$) as a result of canning (Table 3). Previous research has shown
357 that heat-processed fish provided higher *b** values than raw fish,[33] in concurrence with
358 the present results. In fact, a direct association between *b** values and lipid oxidation
359 development was proven.[46] A comparison among the canned samples showed lower *b**
360 values ($p<0.05$) in canned muscle packed with the two highest alga contents.
361 Consequently, an inhibitory effect on the *b** value increase was implied by the alga
362 presence in the packing medium. Such conclusion is in agreement with the above-
363 mentioned results concerning the assessment of lipid oxidation development.

364

365 **CONCLUSIONS**

366 The inclusion of an aqueous *B. bifurcata* extract in the packing system has led to a
367 quality enhancement of canned Atlantic mackerel. This effect was intensified by
368 increasing the alga extract presence. Thus, an inhibitory effect on the lipid oxidation
369 development (tertiary compounds formation) and on color parameters (*L** and *b**) was
370 observed due to the alga presence in the packing medium. On the contrary, the presence

371 of the alga extract did not produce any effect on the volatile compounds (total and
372 trimethylamine) formation and the lipid hydrolysis (free fatty acids formation)
373 development.

374 The packing system proposed in this work constitutes a novel and promising
375 strategy to enhance the quality of commercial canned fish products. In order to optimize
376 the experimental conditions, further research is envisaged, to analyze molecules
377 involved in the present biopreservation, as well as their stability and behavior under
378 thermal treatment.

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REFERENCES

1 Aitken A, Connell J, Fish, in *Effects of heating on food stuffs*, ed. by Priestley R. Applied Science Publishers Ltd., London, UK, pp. 219-254 (1979).

2 Horner W, Canning fish and fish products, in *Fish Processing Technology*, ed. by Hall G, 2nd edition, Blackie Academic and Professional, Chapman and Hall, London, UK, pp. 119-159 (1997).

3 Aubourg SP, Review: Loss of quality during the manufacture of canned fish products. *Food Sci Technol Internat* **7**:199-215 (2001).

4 Lukoshkina M and Odoeva G, Kinetics of chemical reactions for prediction of quality of canned fish during storage. *App Biochem Microb* **39**:321-327 (2003).

5 Romotowska P, Gudjonsdóttir M, Kristinsdóttir T, Karlsdóttir M, Arason S, Jónsson Á and Kristinsson H, Effect of brining and frozen storage on physicochemical properties of well-fed Atlantic mackerel (*Scomber scombrus*) intended for smoking and canning. *LWT-Food Sci Technol* **72**:199-205 (2016).

6 Naseri M and Rezaei M, Lipid changes during long-term storage of canned sprat. *J Aquat Food Prod Technol* **21**:48-58 (2012).

7 Aubourg SP, Gallardo J and Medina I, Changes in lipids during different sterilising conditions in canning albacore (*Thunnus alalunga*) in oil. *Int J Food Sci Technol* **32**:427-431 (1997).

8 Frankel E and Meyer A, The problems of using one-dimensional methods to evaluate multifunctional food and biological antioxidants. *J Sci Food Agric* **80**:1925-1941 (2000).

9 Serrano J, Puupponen-Pimia R, Dauer A, Aura A and Saura-Calixto F, Tannins: Current knowledge of food sources, intake, bioavailability and biological effects. *Molec Nutr Food Res* **53**:S310-S329 (2009).

10 Zubia M, Fabre MS, Kerjean V, Le Lann K, Stiger-Pouvreau V, Fauchon M and Deslandes E, Antioxidant and antitumoral activities of some Phaeophyta from Britanny coasts. *Food Chem* **116**:693-701 (2009).

11 Gupta S and Abu-Ghannam N, Bioactive potential and possible health effects of edible brown seaweeds. *Trends Food Sci Technol* **22**:315-326 (2011).

12 Smit A, Medicinal and pharmaceutical uses of seaweed natural products: A Review. *J Appl Phycol* **16**:245-262 (2004).

13 Paiva L, Lima E, Ferreira Patarra R, Neto A and Baptista J, Edible Azorean macroalgae as source of rich nutrients with impact on human health. *Food Chem* **164**:128-135 (2014).

14 Braune W, *Meeresalgen. Ein Farbbilführer zu den verbreiteten benthischen Grün- Braun- und Rotalgen der Weltmeere.* ARG Gantner Verlag, Ruggel, Liechtenstein, pp. 1-596 (2008).

15 Le Lann K, Jégou C and Stiger-Pouvreau V, Effect of different conditioning treatments on total phenolic content and antioxidant activities in two Sargassacean species: Comparison of the frondose *Sargassum muticum* (Yendo) Fensholt and the cylindrical *Bifurcaria bifurcata* R. Ross. *Phycol Res* **56**:238-245 (2008).

16 Gómez-Ordóñez E, Jiménez-Escrig A and Rupérez P, Dietary fibre and physicochemical properties of several edible seaweeds from the northwestern Spanish coast. *Food Res Internat* **43**:2289-2294 (2010).

17 Glombitza K, Rösener H and Koch M, Polyhydroxyoligophenyle und phenyläther aus *Bifurcaria bifurcata*. *Phytochem* **15**:1279-1281 (1976).

437 18 Culioli G, Daoudi M, Ortalo-Magne A, Valls R and Piovetti L, (S)-12-
438 hydroxygeranylgeraniol-derived diterpenes from the brown alga *Bifurcaria*
439 *bifurcata*. *Phytochem* **57**:529-535 (2001).
440 19 Bouzidi N, Daghbouche Y, El Hattab M, Aliche Z, Culioli G, Piovetti L, Garrigues S
441 and De la Guardia M, Determination of total sterols in brown algae by Fourier
442 transform infrared spectroscopy. *Anal Chim Acta* **616**:185-189 (2008).
443 20 Gómez-Ordóñez E and Rupérez P, FTIR-ATR spectroscopy as a tool for
444 polysaccharide identification in edible brown and red seaweeds. *Food Hydroc*
445 **25**:1514-1520 (2011).
446 21 Miranda J, Trigo M, Barros-Velázquez J and Aubourg SP, Effect of an icing medium
447 containing the alga *Fucus spiralis* on the microbiological activity and lipid
448 oxidation in chilled megrim (*Lepidorhombus whiffiagonis*). *Food Cont* **59**:290-
449 297 (2016).
450 22 Bligh E and Dyer W, A rapid method of total extraction and purification. *Canad J*
451 *Biochem Physiol* **37**:911-917 (1959).
452 23 Lowry R and Tinsley I, Rapid colorimetric determination of free fatty acids. *J Amer*
453 *Oil Chem Soc* **53**:470-472 (1976).
454 24 Kim R and Labella F, Comparison of analytical methods for monitoring autoxidation
455 profiles of authentic lipids. *J Lipid Res* **28**:1110-1117 (1987).
456 25 Chapman R and McKay J, The estimation of peroxides in fats and oils by the ferric
457 thiocyanate method. *J Amer Oil Chem Soc* **26**:360-363 (1949).
458 26 Aubourg SP and Medina I, Quality differences assessment in canned sardine
459 (*Sardina pilchardus*) by fluorescence detection. *J Agric Food Chem,* **45**:3617-
460 3621 (1997).

461 27 Antonacopoulos N, Verbesserte Apparatus zur quantitativen Destillation
462 wasserdampfflüchtiger Stoffe. *Zeitt Lebensm Unters Forsch* **13**:113-160 (1960).
463 28 Tozawa H, Erokibara K and Amano K, Proposed modification of Dyer's method for
464 trimethylamine determination in codfish, in *Fish Inspection and Quality Control*,
465 ed. by Kreuzer R. Fishing News Books Ltd., London, UK, pp. 187-190 (1971).
466 29 Selmi S, Monser L and Sadok S, The influence of local canning process and storage
467 on pelagic fish from Tunisia: Fatty acids profile and quality indicators. *J Food*
468 *Process Preserv* **32**:443–457 (2008).
469 30 Ortiz J, Vivanco JP and Aubourg SP, Lipid and sensory quality of canned Atlantic
470 salmon (*Salmo salar*): Effect of the use of different seaweed extracts as covering
471 liquids. *Eur J Lipid Sci Technol* **116**:596-605 (2014).
472 31 Howell N, Interaction of proteins with small molecules, in *Ingredient interactions*,
473 ed. by Gaonkar A. Marcel Dekker, New York, USA, pp. 269-289 (1995).
474 32 Tironi V, Tomás M and Añón MC, Structural and functional changes in myofibrillar
475 proteins of sea salmon (*Pseudopercis semifasciata*) by interaction with
476 malondialdehyde (RI). *J Food Sci* **67**:930-935 (2002).
477 33 Rodríguez A, Carriles N and Aubourg SP, Effect of chill storage under different
478 icing conditions on sensory and physical properties of canned farmed salmon
479 (*Oncorhynchus kisutch*). *Int J Food Sci Technol* **45**:295-304 (2010).
480 34 Connan S, Deslandes E and Gall E, Influence of day-night and tidal cycles on phenol
481 content and antioxidant capacity in three temperate intertidal brown seaweeds. *J*
482 *Experim Mar Biol Ecol* **349**:359-369 (2007).
483 35 Wang T, Jónsdóttir R and Olafsdóttir G, Total phenolic compounds, radical
484 scavenging and metal chelation of extracts from Icelandic seaweeds. *Food Chem*
485 **116**:240-248 (2009).

36 Farvin K and Jacobsen C, Phenolic compounds and antioxidant activities of selected species of seaweeds from Danish coast. *Food Chem* **138**:1670-1681 (2013).

37 Athukorala Y, Lee KW, Song C, Ahn CB, Shin TS, Cha YJ, Shahidi F and Jeon YJ, Potential antioxidant activity of marine red alga *Grateloupia filicina* extracts. *J Food Lipids* **10**:251-265 (2003).

38 Tierney M, Smyth T, Hayes M, Soler-Vila A, Croft A and Brunton N, Influence of pressurised liquid extraction and solid-liquid extraction methods on the phenolic content and antioxidant activities of Irish macroalgae. *Int J Food Sci Technol* **48**:860-869 (2013).

39 Kuda T and Ikemori T, Minerals, polysaccharides and antioxidant properties of aqueous solutions obtained from macroalgal beach-casts in the Noto Peninsula, Ishikawa, Japan. *Food Chem* **112**:575-581 (2009).

40 Pereira L, Amado A, Critchley A, van de Velde F and Ribeiro-Claro P, Identification of selected seaweed polysaccharides (phycocolloides) by vibrational spectroscopy (FTIR-ATR and FT-Raman). *Food Hydroc* **23**:1903-1909 (2009).

41 Medina I, Sacchi R, Biondi L, Aubourg SP and Paolillo L, Effect of packing media on the oxidation of canned tuna lipids. Antioxidant effectiveness of extra virgin olive oil. *J Agric Food Chem* **46**:1150-1157 (1998).

42 Medina I, Sacchi R and Aubourg SP, A ^{13}C-NMR study of lipid alterations during fish canning: Effect of filling medium. *J Sci Food Agric* **69**:445-450 (1995).

43 Gallardo J, Pérez-Martín R, Franco J, Aubourg SP and Sotelo C, Changes in volatile bases and trimethylamine oxide during the canning of albacore (*Thunnus alalunga*). *Int J Food Sci Technol* **25**:78-81 (1990).

509 44 Losada V, Rodríguez A, Ortiz J and Aubourg SP, Quality enhancement of canned
510 sardine (*Sardina pilchardus*) by a preliminary chilling treatment. *Eur J Lipid Sci*
511 *Technol* **108**:598-605 (2006).
512 45 Schubring R, Comparative study of the DSC pattern, color texture and water-binding
513 capacity of rainbow trout muscle during heating. *J Food Proces Preserv* **32**:190-
514 218 (2008).
515 46 Undeland I, Hultin H and Richards M, Aqueous extracts from some muscles inhibit
516 hemoglobin-mediated oxidation of cod muscle membrane lipids. *J Agric Food*
517 *Chem* **51**:3111-3119 (2003).
518

519 **FIGURE LEGENDS**

520

521 **Figure 1**: Assessment of fluorescent compounds formed[§] in canned mackerel muscle
522 packed under various conditions[§§]

523 [§] Average values of five replicates (*n*=5). Standard deviations are indicated by bars.
524 Average values accompanied by different letters (a-d) denote significant
525 differences ($p<0.05$).

526 [§§] Sample abbreviations: PS-0, PS-1, PS-2, PS-3 and PS-4 correspond to packing
527 conditions including 0.000, 0.125, 0.250, 0.625 and 1.250 g of extracted alga in
528 the packing medium, respectively.

529

TABLE 1

Lipid oxidation assessment* in canned mackerel muscle packed under various conditions**

Packing system	Lipid oxidation index***			
	CD	**CT**	**PV**	**PI**
Raw fish	0.69 ab (0.11)	0.03 a (0.01)	0.40 a (0.33)	1.10 a (0.08)
PS-0	0.57 a (0.05)	0.04 ab (0.01)	1.25 a (0.46)	1.13 a (0.09)
PS-1	0.70 ab (0.08)	0.06 b (0.02)	3.20 b (1.46)	1.14 a (0.10)
PS-2	0.72 ab (0.07)	0.05 ab (0.00)	2.49 b (0.28)	1.12 a (0.14)
PS-3	0.75 ab (0.07)	0.06 b (0.01)	8.25 c (3.04)	1.02 a (0.08)
PS-4	0.88 b (0.17)	0.07 b (0.01)	7.67 c (2.18)	1.08 a (0.07)

* Average values of five replicates (*n*=5). Standard deviations are expressed in brackets. Average values followed by different letters (a-c) denote significant differences ($p<0.05$).

** Packing conditions: PS-1, PS-2, PS-3 and PS-4 correspond to canned mackerel including 0.125, 0.250, 0.625 and 1.250 g of extracted alga in the packing medium, respectively. PS-0 denotes canned fish without alga extract in the packing medium (canned control).

*** Indices abbreviations: CD (conjugated dienes), CT (conjugated trienes), PV (peroxide value) and PI (polyene index). CD and CT units as expressed in the Materials and Methods section. PV expressed as meq active oxygen kg^{-1} lipids.

TABLE 2

Free fatty acids (FFA) content and volatile amines (total volatile base-nitrogen TVB-N; trimethylamine-nitrogen, TMA-N) formation* in canned mackerel packed under various conditions**

Packing system	Quality index		
	FFA (g kg^{-1} lipids)	**TVB-N** (mg kg^{-1} muscle)	**TMA-N** (mg kg^{-1} muscle)
Raw fish	1.73 a (0.90)	259.66 c (7.85)	4.19 a (2.52)
PS-0	2.63 a (1.07)	183.09 b (15.22)	25.77 b (2.19)
PS-1	3.87 ab (1.17)	162.56 ab (12.31)	26.97 b (3.07)
PS-2	2.89 ab (0.34)	152.19 a (9.56)	25.80 b (2.90)
PS-3	4.96 b (1.79)	165.78 ab (19.63)	29.00 b (2.32)
PS-4	3.50 ab (0.87)	160.11 ab (18.55)	28.29 b (4.83)

* Average values of five replicates (*n*=5). Standard deviations are expressed in brackets. Average values followed by different letters (a-c) denote significant differences ($p<0.05$).

** Packing conditions as expressed in Table 1.

TABLE 3

Assessment of L* and b* color parameters[§] in canned mackerel muscle packed under various conditions[§§]

Packing system	Color parameter	
	L*	b*
Raw fish	45.63 a (2.41)	1.03 a (0.28)
PS-0	71.40 e (2.10)	17.54 cd (0.62)
PS-1	66.36 d (2.70)	19.43 d (0.96)
PS-2	56.63 c (1.68)	15.52 c (1.42)
PS-3	51.68 b (2.47)	10.29 b (1.50)
PS-4	43.63 a (1.76)	10.10 b (1.85)

[§] Average values of five replicates (*n*=5). Standard deviations are expressed in brackets. Average values followed by different letters (a-e) denote significant differences ($p<0.05$).

[§§] Packing conditions as expressed in Table 1.

Figure 1

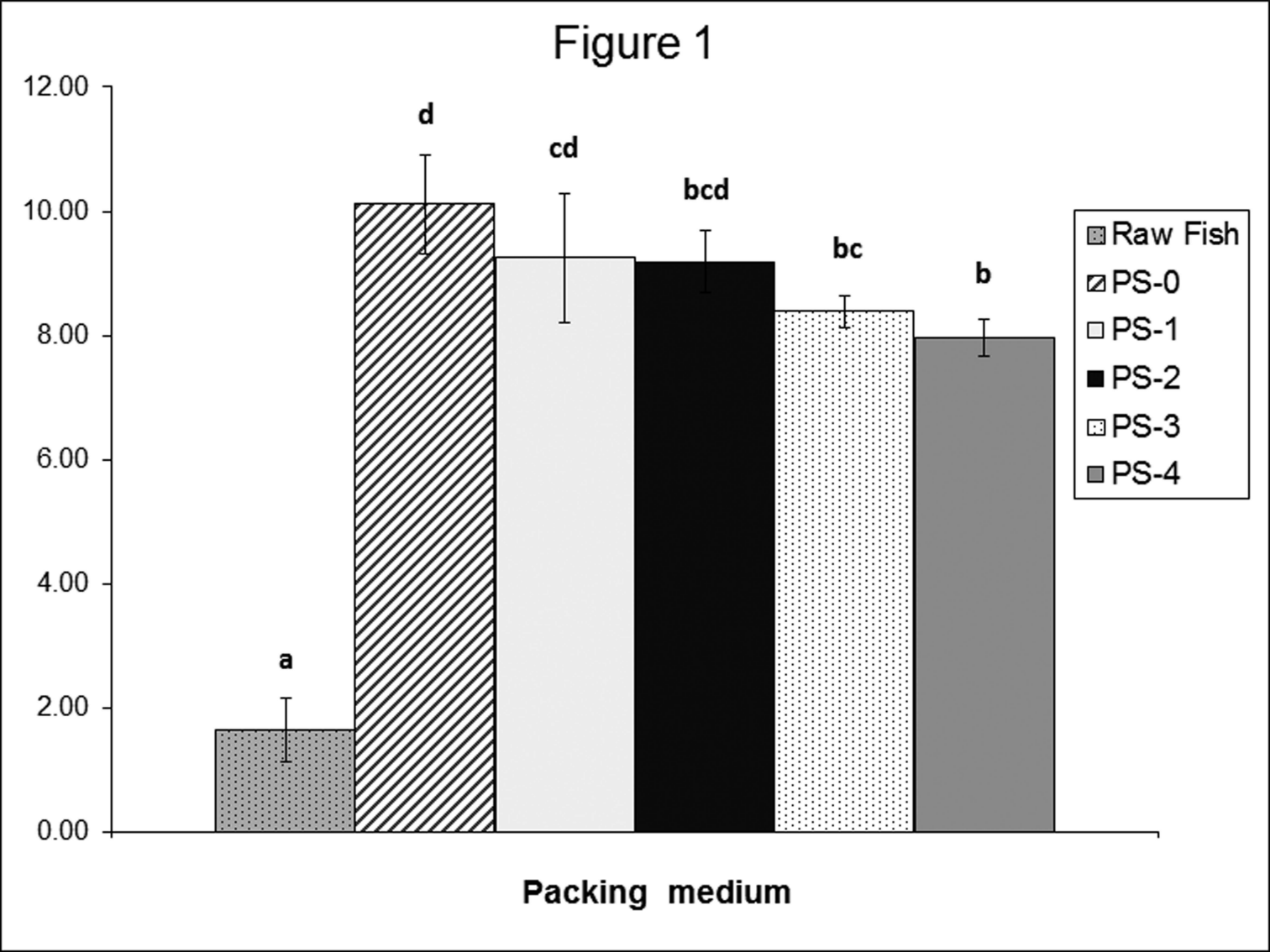

12.00
10.00
8.00
6.00
4.00
2.00
0.00
a
d
cd
bcd
bc
b
Raw Fish
PS-0
PS-1
PS-2
PS-3
PS-4
Packing medium

Figure 2

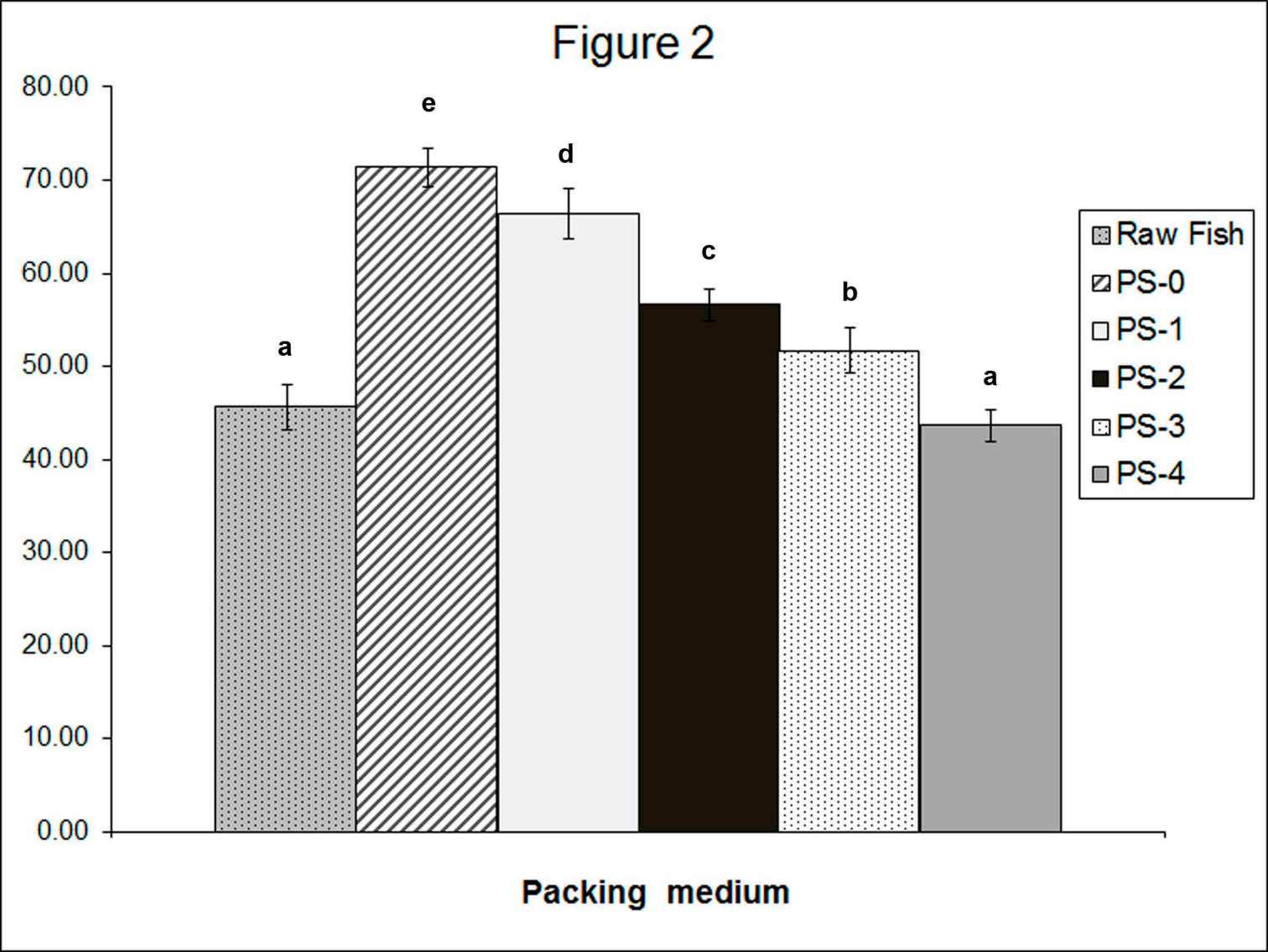
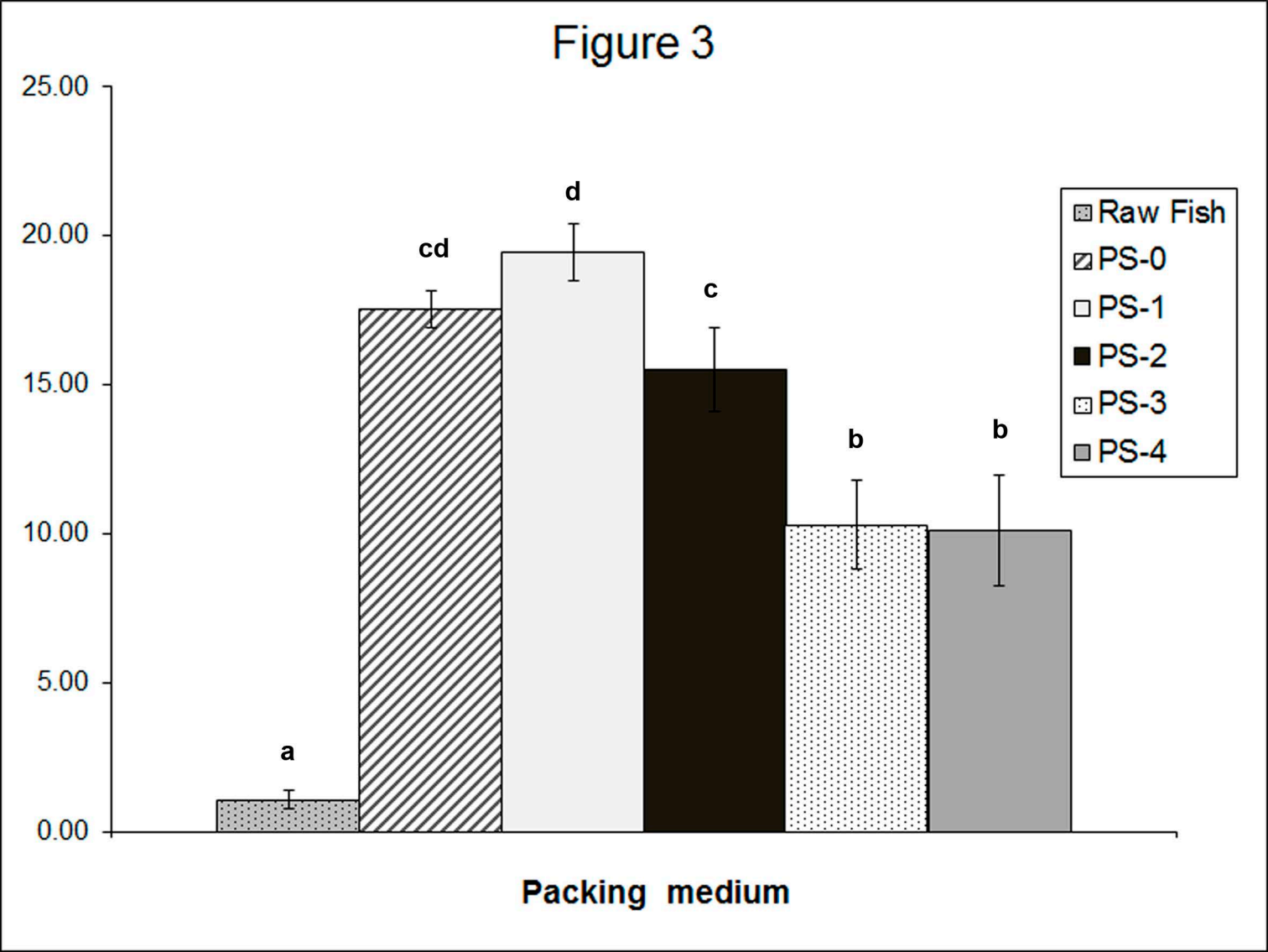

80.00
70.00
60.00
50.00
40.00
30.00
20.00
10.00
0.00
a
e
d
c
b
a
Raw Fish
PS-0
PS-1
PS-2
PS-3
PS-4
Packing medium

Figure 3


25.00
20.00
15.00
10.00
5.00
0.00
a
cd
d
c
b
b
Raw Fish
PS-0
PS-1
PS-2
PS-3
PS-4
Packing medium