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Antioxidant effect of an aqueous extract of alga *Cystoseira stricta* during the frozen storage of Atlantic Chub mackerel (*Scomber colias*)

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An aqueous extract of alga *Cystoseira stricta* was included in the glazing medium employed during the frozen storage of Atlantic Chub mackerel (*Scomber colias*). Rancidity stability of frozen fish muscle was determined throughout a 9-month storage at –18 ºC. An inhibitory effect on the development of lipid oxidation (assessment of peroxides, thiobarbituric acid and fluorescence indices) was observed as a result of the alga extract presence in the glazing system; thus, a marked retention of polyunsaturated fatty acids and alpha-tocopherol contents was achieved. Furthermore, an inhibitory effect on the lipid hydrolysis development and trimethylamine formation was implied as a result of the alga extract presence. Interestingly, enhancement of rancidity stability in frozen mackerel was found stronger by increasing the concentration of the alga extract in the glazing medium. A preservative effect of aqueous alga extract is established, this effect being attributed to the presence of potential active compounds able to stabilise radicals responsible for the lipid oxidation development.

Keywords: *Cystoseira stricta*; aqueous extract; frozen fish; glazing; rancidity; quality.

INTRODUCTION

The presence in fish species of a highly unsaturated lipid composition and the high content of pro-oxidant molecules have been reported as the most decisive factors influencing the shelf-life of frozen fatty fish products [1]. To retard lipid oxidation as long as possible and, accordingly, extend the shelf life, a wide number of advanced and traditional strategies to be combined to freezing and frozen storage have been tested such as hydrostatic high pressure, vacuum packaging, glazing, preservatives addition and active and intelligent packaging [2-5].

Marine algae have widely been consumed as food in Asian countries for centuries. As photosynthetic organisms, algae are known to be exposed to a combination of light and high oxygen concentration. The lack of structural damage in their organs has led to consider that their protection against oxidation would arise from their natural content on antioxidant substances [6, 7]. Consequently, marine algae are receiving an increasing attention as a source of bioactive compounds (i.e., polyphenols, carotenoids, etc.) able to inhibit lipid oxidation development during food processing [8-10].

Among brown macroalgae, *Cystoseira* genus has shown to be widely distributed in temperate regions of the Northern hemisphere such as the Mediterranean Sea, and the Indic and Pacific Oceans. Interestingly, several *in vitro* studies reported the preservative effect of certain bioactive compounds present in such algae. Thus, an antioxidant effect of *C. tamarisfolia* [11], *C. compressa* [12] and *C. hakodatensis* [13] extracts has been reported, this effect being explained by the presence of potential active compounds such as pholoroglucinol, manitol, fatty acids, fucosterol and polyphenols in general.

The current research focused on *C. stricta*. Thus, previous research showed the presence in this alga of profitable chemical constituents such as polyunsaturated fatty acids (PUFA), macro- and micro-mineral elements, as well as a relevant alpha-tocopherol content [14]. In this study, an aqueous extract of this alga was included in the glazing medium employed during the frozen storage of Atlantic Chub mackerel (*Scomber colias*). Its effect on lipid damage development was measured throughout a 9-month frozen storage (–18 ºC) in mackerel muscle.

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MATERIALS AND METHODS

Preparation of algae extracts and glazing systems

Fresh *C. stricta* was collected in May 2016 on the Oran coast, Western Algeria (35° 44′ 29.72″ N and 0° 50′ 14.21″ W). Species identification was made in the Laboratory of Aquaculture and Bioremediation by employing the Algabase site (www.algabase.com) [15]. Upon arrival to the laboratory, alga samples were washed thoroughly with running water to remove salts, sand and epiphytes. Biomass was then washed with distilled water and dried at room temperature (23 ± 2 °C) for 72 h in the dark. Then, the dried material was milled, powdered and stored at −20 °C until further analysis.

To prepare the glazing systems, 100 g of dried alga were mixed with distilled water (1,600 mL), stirred for 30 s, centrifuged at 3,500 rpm for 10 min at 4 ºC and the supernatant recovered. This procedure was carried out two more times. The collected extracts were pooled together and adjusted to 5 L with distilled water.

Three different concentrations of the alga extract were tested as glazing medium. For it, 385, 1,153 and 3,461 mL (corresponding to 7.7, 23.1 and 69.2 g of dried alga, respectively) of the alga extract were adjusted to 11 L by addition of distilled water, respectively. The resulting solutions were employed as glazing systems and labelled as G-1, G-2 and G-3 conditions, respectively.

Fish material, processing and sampling

Fresh Atlantic Chub mackerel (153 specimens) were caught near the Galician Atlantic coast (North-Western Spain) and transported in ice to the laboratory. The length and weight of the fish specimens ranged from 24.5 to 27.5 cm and from 105 to 123 g, respectively.

Upon arrival to the laboratory, nine specimens (three groups of three individuals each) were separated and analysed independently (n = 3). The remaining fish specimens were divided into four batches (36 individuals in each batch) that were immediately frozen at –40 ºC. After 48 hours at –40 ºC, one batch was immersed in water, while the three others were immersed in the G-1, G-2 and G-3 systems, respectively. In all cases, fish specimens were immersed for 30 s at 0 ºC, allowed to drain for 15 s, packaged in polyethylene bags (three pieces per bag) and stored at –18 ºC. Sampling was undertaken at months 1, 3, 6 and 9 of frozen storage at –18 ºC. At each time and for each condition, nine individuals were taken, that were divided into three groups (three individuals per group) and studied separately. Analysis of frozen material was undertaken after thawing; thawing was carried out by overnight storage in a cool room (4 ºC).

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

Chemical analyses related to quality loss

Lipids were extracted from the mackerel white muscle by the Bligh and Dyer [16] method, which employs a single-phase solubilisation of the lipids using a chloroform-methanol (1:1) mixture.

Peroxide value (PV) was determined spectrophotometrically (520 nm) (Beckman Coulter DU 640 spectrophotometer) on the lipid extract of the fish muscle by peroxide reduction with ferric thiocyanate, according to Chapman and McKay [17]. The results were calculated as meq. active oxygen·kg^{-1} lipids.

Thiobarbituric acid index (TBA-i) was determined according to Vyncke [18]. This method is based on the reaction between a trichloroacetic acid extract of the fish muscle and thiobarbituric acid (TBA). 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 aqueous phase obtained during the lipid extraction [16] of the fish muscle. As described previously [19], 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 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, being then analysed by gas–liquid chromatography (Perkin Elmer 8700 chromatograph, Madrid, Spain) [20]. Peaks corresponding to FAME were identified by comparison of their retention times with those of standard mixtures (Qualmix Fish, Larodan, Malmo, Sweden; FAME Mix, Supelco, Inc.). Peak areas were automatically integrated; C19:0 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.

The profile of tocopherol compounds was analysed according to the method of Cabrini *et al*. [21]. For this, mackerel muscle was extracted with hexane, which was eliminated under nitrogen flux. The resulting alga extracts were then dissolved in isopropanol and injected into an HPLC system (ODS column, 15 cm × 0.46 cm i.d.); detection was achieved at 280 nm. The presence of different tocopherol compounds (alpha, beta, gamma and delta) was checked, their content being calculated with calibration curves prepared from the corresponding commercial tocopherols. For each tocopherol compound, results were expressed as $mg \cdot kg^{-1}$ fish muscle.

Free fatty acid (FFA) content was determined using the lipid extract of the fish muscle by the Lowry and Tinsley [22] method, which is based on complex formation with cupric acetate-pyridine followed by spectrophotometric (715 nm) assessment. The results were expressed as mg $FFA \cdot kg^{-1}$ muscle.

Trimethylamine-nitrogen (TMA-N) values were determined using the picrate colorimetric method, as previously described by Tozawa *et al*. [23]. This method involved the preparation of a 5% trichloroacetic acid extract of fish muscle (10 g/25 mL). Results were calculated as mg $TMA\text{-}N \cdot kg^{-1}$ muscle.

Statistical analysis

Data obtained from all chemical analyses were subjected to the ANOVA method to explore differences resulting from the effect of the glazing system. The comparison of means was performed using the least-squares difference (LSD) method. In all cases, analyses were carried out using the PASW Statistics 18 software for Windows (SPSS Inc., Chicago, IL, USA); differences among glazing batches were considered significant for a confidence interval at the 95 % level ($p < 0.05$) in all cases.

RESULTS AND DISCUSSION

Lipid oxidation development

This damage mechanism was measured by assessing the primary (peroxides), secondary (TBARS) and tertiary (fluorescence ratio) lipid oxidation compounds, as well as by the determination of the polyene index.

Peroxides formation showed a progressive increase with frozen storage time in all kinds of samples (Table 1). Comparison among batches revealed an inhibitory effect on primary oxidation as a result of the presence of the alga extract in the glazing system. Thus, lower average values could be observed in fish corresponding to alga-glazing batches when compared to their counterparts from the Control throughout the 3-9-month period. Differences were found significant ($p < 0.05$) at months 3 and 9 (G-3 batch) and at month 9 (G-2 batch).

Formation of TBARS also showed a progressive increase with time in all batches (Table 1).

Lower average values were obtained during the 3-9-month period for fish corresponding to G-2 and G-3 batches when compared to their counterparts from Control and G-1 batches; however, scarce significant differences ($p > 0.05$) could be obtained.

A slight increasing tendency of fluorescent compounds was observed in all kinds of batches under study (Table 1). Interestingly, lower average values throughout the 1-9-month period were implied for fish samples belonging to G-2 and G-3 batches when compared to their counterparts from Control and G-1; differences were found significant ($p < 0.05$) at months 3 and 6 (G-3 batch) and month 1 (G-2 batch). As for primary and secondary lipid oxidation compounds, no significant differences ($p > 0.05$) could be observed between G-2 and G-3 batches.

Finally, the PI assessment showed a general loss in all batches with storage time (Table 2), which agrees to the above-mentioned progressive increase of the content of all kinds of oxidation compounds. Also in agreement to such data, higher average values were observed in samples including any of the concentrations tested of the alga extract in the glazing system when compared to the Control; such differences were found significant ($p < 0.05$) for G-3 (3-9-month period) and G-2 (month 3) batches.

Frozen storage is known to be associated with fish lipid oxidation processes where different kinds of endogenous enzymes may be involved [24]. Inhibition of lipid oxidation found in the current research can be explained by the presence of antioxidant compounds in the aqueous extract of the current alga. Thus, although alcoholic solvents have been preferred as being the most accurate to obtain a high total phenolic content [6, 8], water extraction of algae has been reported to produce in most cases the highest yields, this including the majority of water-soluble compounds with preservative effects such as polysaccharides, proteins, glycosides and peptides [7, 9, 10].

Table 1. Assessment of lipid damage* in frozen mackerel glazed under different conditions**

Quality index	Storage time (months)	Glazing medium			
		Control	G-1	G-2	G-3
Peroxide value (meq. active oxygen·kg^{-1} lipids)	Initial	2.93 (1.15)			
	1	5.53 a (0.99)	4.42 a (1.53)	5.25 a (1.02)	5.61 a (1.29)
	3	9.20 b (0.47)	9.19 ab (3.94)	8.19 ab (1.51)	7.15 a (1.29)
	6	9.71 a (1.18)	9.05 a (1.70)	8.19 a (1.51)	6.98 a (1.77)
	9	19.92 b (2.62)	19.26 b (1.15)	12.30 a (0.96)	9.19 a (1.85)
Thiobarbituric acid index (mg malondialdehyde kg^{-1} muscle)	Initial	0.27 (0.07)			
	1	0.49 a (0.07)	0.50 a (0.03)	0.60 a (0.07)	0.56 a (0.12)
	3	0.86 b (0.18)	0.74 ab (0.14)	0.58 a (0.10)	0.64 ab (0.04)
	6	0.87 ab (0.23)	0.92 b (0.18)	0.54 ab (0.20)	0.52 a (0.15)
	9	1.22 a (0.02)	1.36 a (0.22)	1.12 a (0.11)	0.91 a (0.27)
Fluorescence ratio	Initial	0.49 (0.07)			
	1	0.81 b (0.16)	0.73 b (0.06)	0.58 a (0.02)	0.61 ab (0.09)
	3	0.82 b (0.01)	0.82 b (0.14)	0.77 ab (0.04)	0.75 a (0.01)
	6	0.91 b (0.05)	0.93 b (0.07)	0.80 ab (0.18)	0.79 a (0.04)
	9	1.32 a (0.10)	1.24 a (0.12)	1.23 a (0.11)	1.19 a (0.14)

* Average values of three replicates (n = 3); standard deviations are indicated in brackets. For each frozen storage time, values accompanied by different letters (a, b) indicate significant differences ($p < 0.05$); ** Glazing conditions: Control (water glazing, without alga extract), G-1 (low content of alga extract), G-2 (medium content of alga extract) and G-3 (high content of alga extract) in agreement to the Material and Methods section.

In agreement with the present study, previous related research accounts for lipid oxidation inhibition in canned fish by including a water extract of *Durvillaea Antarctica*, *Pyropia columbina* and *Ulva lactuca* [25] and of *Fucus spiralis* and *U. lactuca* [26] in the packaging medium. Related to fresh fish, the presence of a combined aqueous-ethanolic extract of *Fucus spiralis* in the icing medium employed during chilling storage of European hake (*Merlucccius merlucius*) led to an increased rancidity inhibition [27]. Furthermore, the presence of Pollock (*Theragra chalcogramma*) skin hydrolysates in frozen (4 months at –35 ºC) pink salmon [3] led to a lower TBARS formation, while the inclusion of aqueous/ethanolic extracts of *F. spiralis* and *Bifurcaria bifurcata* in the glazing system led to a lower development of rancid odour and taste in frozen (8 months at –18 ºC) mackerel (*S. colias*) [5]. Concerning the analysis of tocopherol compounds, only alpha-tocopherol was detected in mackerel muscle in the present study. Such result agrees to previous studies on wild fish species [26]. Contrary, farmed fish species have shown the presence of different kinds of tocopherol compounds, according to the composition of their diet [28]. In the current study, a marked loss of alpha-tocopherol was evident as a result of the frozen storage (Table 2). However, this loss was partially inhibited by the presence in the glazing system of the alga extract. Thus, higher average values were observed throughout the whole storage period for any of the alga-glazing batches when compared to the Control; however, significant differences ($p < 0.05$) were only obtained at month 3 for fish corresponding to the G-2 batch.

Table 2. Assessment of the polyene index and alpha-tocopherol content* in frozen mackerel glazed under different conditions**

Quality index	Storage time (months)	Glazing medium			
		Control	G-1	G-2	G-3
Polyene index	Initial	2.83 (0.12)			
	1	2.68 a (0.23)	2.68 a (0.12)	2.92 a (0.16)	2.86 a (0.07)
	3	2.56 a (0.08)	2.69 a (0.08)	2.75 b (0.07)	2.74 ab (0.14)
	6	2.38 a (0.10)	2.43 a (0.07)	2.48 a (0.07)	2.56 a (0.07)
	9	2.23 a (0.19)	2.41 a (0.21)	2.48 a (0.17)	2.51 a (0.09)
Alpha-tocopherol content (mg·kg^{-1} fish muscle)	Initial	39.59 (5.79)			
	1	24.05 a (3.80)	30.53 a (6.42)	28.98 a (1.66)	31.68 a (7.91)
	3	27.87 a (1.38)	30.01 ab (3.38)	30.43 b (0.83)	35.46 b (5.45)
	6	22.44 a (3.55)	23.48 ab (5.26)	28.63 ab (5.18)	29.75 b (3.02)
	9	12.61 a (4.59)	18.83 ab (5.66)	19.94 ab (3.21)	26.81 b (0.63)

* Average values of three replicates (n = 3); standard deviations are indicated in brackets. For each frozen storage time, values accompanied by different letters (a, b) indicate significant differences (p < 0.05). ** Glazing conditions as expressed in Table 1.

Table 3. Evolution of free fatty acids (FFA) and trimethylamine (TMA) formation* in frozen mackerel glazed under different conditions**

Quality index	Storage time (months)	Glazing medium			
		Control	G-1	G-2	G-3
FFA (mg·kg^{-1} muscle)	Initial	40.27 (0.19)			
	1	315.95 a (16.26)	336.19 a (9.86)	347.00 a (29.21)	325.17 a (4.79)
	3	484.4 b (15.40)	464.15 ab (6.20)	458.01 ab (11.41)	447.32 a (9.29)
	6	722.05 b (7.73)	658.06 a (4.79)	657.03 a (12.03)	641.86 a (4.73)
	9	1,081.41 d (12.87)	983.23 c (11.68)	905.66 b (12.42)	848.49 a (6.72)
TMA value (mg TMA-N·kg^{-1} muscle)	Initial	1.75 (0.13)			
	1	5.94 b (1.28)	3.94 ab (0.47)	4.42 b (0.44)	3.53 a (0.18)
	3	7.25 b (0.78)	6.08 a (0.10)	7.01 b (0.65)	6.76 b (0.21)
	6	10.67 a (1.77)	8.73 a (0.71)	8.41 a (1.21)	9.26 a (0.58)
	9	14.21 b (0.22)	12.89 ab (1.25)	11.87 a (0.18)	11.90 a (1.28)

* Average values of three replicates (n = 3); standard deviations are indicated in brackets. For each frozen storage time, values accompanied by different letters (a, b, c, d) indicate significant differences (p < 0.05). ** Glazing conditions as expressed in Table 1.

It is concluded that a preservative effect on the alpha-tocopherol content has been produced during the frozen storage time as a result of the presence of the antioxidant compounds present in the alga extract. This result agrees with the above-mentioned lower levels in oxidation indices previously described. A protective effect on alpha-tocopherol content was also observed in canned fish by the presence of a water extract of different kinds of algae such as *Durvillaea Antarctica, Pyropia columbina* and *U. lactuca* [25] and of *F. spiralis* and *U. lactuca* [26] in the packaging medium.

Formation of FFA and TMA

A very strong formation of FFA was implied throughout the frozen storage in all kinds of samples (Table 3).

However, the presence in the glazing system of the alga extract led in all cases to lower average values in the 3-9-month period when compared to the Control; interestingly, a significant effect ($p < 0.05$) was implied in the 3-9-month period (G-3 batch) and in the 6-9-month period (G-2 and G-1 batches). At the end of the study, fish corresponding to the G-3 batch showed lower ($p < 0.05$) FFA values than any other batch, so that a positive effect of the alga extract content in the glazing medium could be concluded.

Lipid hydrolysis development has been recognised as a most important event during the frozen storage of fish species [19, 28]. This degradative pathway has been signalled during the frozen storage as the result of an increased hydrolytic endogenous enzyme (lipases, phospholipases) activity by lipases release from liposomes in muscle, which then facilitates closer proximity between enzyme and substrate [24].

A marked formation of TMA was detected in all samples throughout the whole study (Table 3). At all sampling times, fish corresponding to Control condition provided higher average values than their counterparts of the different alga-glazing batches. Differences were found significant ($p < 0.05$) at months 1 and 9 (G-3 batch), month 9 (G-2 batch) and month 3 (G-1 batch). Consequently, an inhibitory effect on the formation of this amine could be concluded by the presence of an extract of the current alga in the glazing system.

During fish processing in general, TMA formation has been explained on the basis of microbial activity (i.e., breakdown of trimethylamine N-oxide, TMAO) [29] and protein degradation [30]. Since a frozen storage process is encountered in the current study, microbial activity ought to be minimised so that protein breakdown should be the main pathway for TMA formation. According to the lower TMA formation in the current study, it could be implied that a protective effect on protein degradation has been produced by the presence in the glazing medium of *C. stricta* extract.

CONCLUSIONS

An increase of rancidity stability in frozen mackerel was observed on the basis of the determination of lipid oxidation indices (peroxides, TBA and fluorescence indices), as well as by the protective effect observed on PUFA and alpha-tocopherol contents. Furthermore, an inhibitory effect on lipid hydrolysis development and TMA formation was implied as a result of the alga extract presence in the glazing system. Interestingly, this preservative effect was found stronger by increasing the concentration of alga extract in the glazing medium. A profitable effect of the aqueous extract of the current alga *C. stricta* is concluded. Further research is envisaged to analyse molecules involved in this preservative action.

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REFERENCES

1. A. Kolakowska, in: Chemical and functional properties of food lipids, Z. Sikorski, A. Kolakowska (eds.), CRC Press, London, UK, 2003, p. 133.
2. A. López-Rubio, R. Gavara, J. Lagarón, *Trends Food Sci. Technol.*, **17**, 567 (2006).
3. S. Sathivel, J. Huang, P. Bechtel, *J. Food Biochem*., **32**, 247 (2008).
4. S. P. Aubourg, *Int. J. Food Sci. Technol.,* **53**, 873 (2018).
5. M. Trigo, M. López, G. Dovale, J. Ortiz, A. Rodríguez, S. P. Aubourg, *Bulg. Chem. Comm*., **51**, 216 (2019).
6. T. Wang, R. Jonsdóttir, G. Olafsdóttir, *Food. Chem*., **116**, 240 (2009).
7. I. Peinado, J. Girón, G. Koutsidis, J. M. Ames, *Food Res. Int*., **66**, 36 (2014).
8. K. Farvin, C. Jacobsen, *Food Chem*., **138**, 1670 (2013).
9. M. Tierney, T. Smyth, M. Hayes, A. Soler-Vila, A. Croft, N. Brunton, *Int. J. Food Sci. Technol*., **48**, 860 (2013).
10. J. Fleurence, M. Morançais, J. Dumay, P. Decottignies, V. Turpin, M. Munier, N. García-

Bueno, P. Jaouen, *Trends Food Sci. Technol.*, **27**, 57 (2012).
11. M. Zubia, M. S. Fabre, V. Kerjean, K. Le Lann, V. Stiger-Pouvreau, M. Fauchon, E. Deslandes, *Food Chem*., **116**, 693 (2009).
12. H. Oucif, J. M. Miranda, S. Ali Mehidi, S.-M. Abi-Ayad, J. Barros-Velázquez, S. P. Aubourg, *Eur. Food Res. Technol*., **244**, 291 (2018).
13. M. K. Airanthi, M. Hosokawa, K. Miyashita, *J. Food Sci*., **76**, C104 (2011).
14. H. Oucif, M. Benaissa, S. Ali Mehidi, R. Prego, S. P. Aubourg, S.-M. Abi-Ayad, *J. Aq. Food Prod. Technol.*, **29**, 90 (2020).
15. M. D. Guiry, G. M. Guiry, *AlgaeBase*. National University of Ireland, Galway. http://www.algaebase.org; searched on 05 January 2017.
16. E. Bligh, W. Dyer, *Can. J. Biochem. Physiol.*, **37**, 911 (1959).
17. R. Chapman, J. McKay, *J. Amer. Oil Chem. Soc.*, **26**, 360 (1949).
18. W. Vyncke. *Fette Seifen Anstrichm.*, **72**, 1084 (1970).
19. S. P. Aubourg, H. Lago, N. Sayar, R. González, *Eur. J. Lipid Sci. Technol*., **109**, 608 (2007).
20. V. Álvarez, I. Medina, R. Prego, S. P. Aubourg, *Eur. J. Lipid Sci. Technol*., **111**, 957 (2009).
21. L. Cabrini, L. Landi, C. Stefanelli, V. Barzanti, A. Sechi, *Comp. Biochem. Physiol. Part B Biochem. Mol. Biol.*, **101**, 383 (1992).
22. R. Lowry, I. Tinsley, *J. Amer. Oil Chem. Soc.*, **53**, 470 (1976).
23. H. Tozawa, K. Erokibara, K. Amano, in: Fish Inspection and Quality Control, R. Kreuzer (ed), Fishing News Books Ltd., London, UK, 1971, p. 187.
24. Z. Sikorski, E. Kolakoski, in: Seafood enzymes, N. Haard, B. Simpson (eds.), Marcel Dekker, New York, USA, 2000, p. 451.
25. J. Ortiz, J. P. Vivanco, S. P. Aubourg, *Eur. J. Lipid Sci. Technol.*, **116**, 596 (2014).
26. R. G. Barbosa, M. Trigo, C. Campos, S. P. Aubourg, *Eur. J. Lipid Sci. Technol.* **121**, 1900129 (2019).
27. J. Barros-Velázquez, J. M. Miranda, J. M. Ezquerra-Brauer, S. P. Aubourg, *Int. J. Food Sci. Technol.*, **51**, 2081 (2016).
28. A. Rodríguez, J. M. Cruz, P. Paseiro, S. P. Aubourg, *Food Bioprocess Technol.*, **5**, 2602 (2012).
29. M. Sivertsvik, W. Jeksrud, T. Rosnes, *Int. J. Food Sci. Tecnol*., **37**, 107 (2002)
30. Z. Sikorski, A. Kolakowska, in: Seafood proteins, Z. Sikorski, B. Sun Pan, F. Shahidi (eds), Chapman and Hall, New York, USA, 1994, p. 99.