Bulgarian Chemical Communications, Volume 51, Special Issue A (pp. 137 - 143) 2019

Effect of alga *Undaria pinnatifida* ("wakame") extract on the quality evolution of chilled megrim (*Lepidorhombus whiffiagonis*)

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Received March 15, 2019; Revised April 10, 2019

The current study represents an attempt to apply the alga *Undaria pinnatifida* for the quality enhancement of fish products. For it, ethanolic extracts of this alga were included in the icing system during the chilling storage of megrim (*Lepidorhombus whiffiagonis*). Lipid damage and microbial activity were analysed in megrim muscle after 0, 2, 6 and 9 days. An inhibitory effect ($p < 0.05$) on lipid hydrolysis (free fatty acids assessment) development could be observed as a result of the alga presence in the icing medium. In this study, a low formation of primary (i.e., peroxides) and secondary (thiobarbituric acid reactive substances) lipid oxidation compounds was inferred in both control and treated fish samples. However, determination of fluorescent compounds (tertiary lipid oxidation compounds) revealed lower average scores for treated megrim at advanced storage periods (6-9 days), so that a partial inhibition of lipid oxidation development could be concluded as a result of the presence of the alga extract in the icing medium. Compositional analyses revealed a polyphenol content of 11±1 mg gallic acid equivalent g^{-1} of lyophilised alga and an antioxidant capacity of 27 % (DPPH assay). Interestingly, aerobes, proteolytic and lipolytic bacteria growth was partly slowed down as compared to their control counterparts. Consequently, the proposed novel employment of *U. pinnatifida* can be considered a promising strategy to enhance seafood quality.

Keywords: Wakame; *Lepidorhombus whiffiagonis*; chilling; lipid hydrolysis; lipid oxidation; microbial activity

INTRODUCTION

Flatfish (flounder, sole, turbot, plaice, halibut, etc.) products represent a very important seafood group. Among them, megrim (*Lepidorhombus whiffiagonis*) is considered one of the most relevant commercial species from the Grand Sole North Atlantic fishing bank, being exploited by a large number of European countries. Due to the fast post-mortem deterioration of fish species [1, 2], long-term travel of trawlers require a continuous optimisation of icing systems to meet the increasing consumer demand for high quality and safe fresh products. With this purpose in mind, a variety of preservation strategies have been combined with flake ice. Thus, recent studies accounted for the incorporation of natural preservatives such as extracts of plants belonging to the Lamiaceae family (i.e., thyme, rosemary and oregano) [3-5], natural low-molecular weight organic acids [6, 7] and algae extracts [8, 9] in the icing medium.

Marine algae are well known to be part of the human diet in different Asian countries and constitute a source of beneficial nutrients, such as vitamins, trace minerals, lipids, amino acids and dietary fibres [10, 11]. Recently, red, green and brown macroalgae have offered the possibility of exploring a wide variety of natural compounds with potential antioxidant [12] and antimicrobial [13] activities, susceptible to be applied in the preservation of seafood. Among them, *Undaria pinnatifida*, a brown seaweed reaching an overall length to about 60-100 cm, is being used for human nutrition as the "wakame" product. This alga, native to the Japan Sea and acclimated in a variety of localities all over the world, has shown a rapid expansion and potential invasive risk in the Southwest Atlantic [14]. Interestingly, wakame has recently attracted great attention for its antioxidant and antimicrobial properties [15, 16], as well as for its valuable nutritional support [17] and prohealth effect [18]. Additionally, it has been employed to improve quality characteristics of different kinds of foods such as pasta [19], chocolate [20] and pound cake [21]. However, no previous research has been carried out related to its potential usefulness for the preservation of seafood.

The current study represents to our knowledge the first attempt to apply *U. pinnatifida* for the quality enhancement of refrigerated fish during storage. For it, ethanolic extracts of this alga were included in the icing system during the chilled storage of megrim. The potential inhibitory effects of alga extract on lipid hydrolysis and autoxidation

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as well as its microbial activity were evaluated during a 9-day storage period.

MATERIAL AND METHODS

Preparation of alga extracts and icing systems

Samples of *U. pinnatifida* were collected by handpicking from the coast of Puerto Madryn (Argentine). The alga specimens were cleaned with tap water to remove salts, epiphytes and other suspended materials. Then, specimens were frozen, lyophilised, ground to powder and stored at −18 °C. To prepare the alga extract, three g of lyophilised alga were mixed with absolute ethanol (2 x 12 mL), stirred for 30 s and centrifuged at 3,500×*g* for 10 min at 4 ºC. Then, the supernatant (20 mL) was recovered and diluted to 6 L with distilled water (0.5 g lyophilised alga·L^{-1} aqueous solution). This solution was packaged in polyethylene bags, kept frozen at –18 ºC and later used as icing system (IS-3, alga extract treatment batch). Additionally, two icing conditions were considered as controls. On one side, traditional ice was prepared from distilled water (6 L), packaged and kept frozen at –18 ºC (IS-1, blank control batch). On the other side, 20 mL of absolute ethanol were diluted to 6 L with distilled water; the mixture was packaged and kept frozen in the same way as the two other ices (IS-2, ethanol control batch). Before addition to individual fish specimens, the different icing systems were ground to obtain flake ice.

Raw fish material, chilling storage and sampling

Fresh megrim specimens (90) were caught near the Galician Atlantic coast (North-Western Spain) and transported to the laboratory. Throughout this process (10 h) the fish specimens were maintained in ice. The length and weight of the fish specimens ranged from 22 to 27 cm and from 107 to 135 g, respectively.

Upon arrival to the laboratory, nine individual fish specimens were separated and analysed to determine initial fish quality (day 0). These fish specimens were divided into three different groups (three specimens per group) that were analysed independently (n = 3). The remaining fish specimens were divided into three batches (27 individuals in each batch), that were placed in independent boxes and directly surrounded by different kinds of ice (IS-1, IS-2 and IS-3 batches, respectively), prepared as previously described. Ice was added at a 1:1 fish:ice ratio and all batches were placed inside a refrigerated room (2±1 ºC). Boxes allowing draining of melted ice were used for fish storage. Fish specimens from all batches were stored for a 9-day period, sampling being carried out on days 2, 6 and 9. At each sampling time, nine specimens were taken from each batch for analysis and divided into three groups (three individuals in each group) that were studied independently (n = 3).

Throughout the whole study, solvents and chemical reagents used were of reagent grade (Merck, Darmstadt, Germany).

Phenolic content and antioxidant activity of U. pinnatifida

The total phenolic content of extracts was assessed by the Folin-Ciocalteu's phenol reagent following the method proposed by Kim *et al*. [22]. Results obtained were expressed as mg gallic acid equivalent (GAE)·g^{-1} lyophilised algae.

The antioxidant activity of the extracts was evaluated by the 2,2-diphenyl-2- picryl-hydrazyl (DPPH) method according to Yen and Chen [23]. Results were expressed as scavenge of the DPPH radical and were determined by the following equation:

$$\%DPPH = \frac{Abs\ control - Abs\ sample}{Abs\ control} x\ 100$$

being *Abs* the absorbance at 517 nm.

Total phenolic content and antioxidant activity determinations were performed in duplicate.

Lipid damage assessment in refrigerated fish

Lipids were extracted from fish muscle by the Bligh and Dyer [24] method, which employs a single-phase solubilisation of the lipids using a chloroform-methanol (1:1) mixture. The results were calculated as g lipid·kg^{-1} muscle.

Free fatty acid (FFA) content was determined in the lipid extract of the fish muscle by the Lowry and Tinsley [25] method based on complex formation with cupric acetate-pyridine followed by spectrophotometric (715 nm) assessment. Results were expressed as g FFA·kg^{-1} lipids.

Peroxide value (PV) was determined spectrophotometrically (Beckman Coulter, DU 640; London, UK) using the lipid extract *via* previous peroxide reduction with ferric thiocyanate according to the Chapman and McKay [26] method. The results were expressed as meq. active oxygen·kg^{-1} lipids.

Thiobarbituric acid index (TBA-i) was determined according to Vyncke [27]. This method is based on the reaction between a trichloracetic 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 expressed as mg malondialdehyde·kg^{-1} muscle.

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

molecules) were measured by fluorescence spectroscopy (Fluorimeter LS 45; Perkin Elmer España; Tres Cantos, Madrid, Spain). In agreement with previous research [28], fluorescence measurements were carried out at 393/463 nm and 327/415 nm in the aqueous phase that resulted from the lipid extraction of fish muscle. The relative fluorescence (RF) was calculated as follows: RF = F/F_{st}, where F was the fluorescence measured at each excitation/emission wavelength pair and F_{st} was the fluorescence intensity of a quinine sulphate solution (1 $\mu g \cdot mL^{-1}$ 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: $FR = RF_{393/463\ nm}/RF_{327/415\ nm}$.

In all cases, chemical analyses were carried out on the white muscle of megrim. All analyses were conducted in triplicate.

Microbial activity assessment

Samples of 10 g of fish white muscle were taken aseptically from chilled fish specimens, mixed with 90 mL of 0.1% peptone water (Merck, Darmstadt, Germany) and homogenised in sterilised stomacher bags (AES, Combourg, France) as previously described [29, 30]. Serial dilutions from the microbial extracts were prepared in 0.1% peptone water.

Total aerobes were investigated by surface inoculation on plate count agar (PCA, Oxoid Ltd., London, UK) after incubation at 30 ºC for 48 h. Enterobacteriaceae were investigated by pour plating using Violet Red Bile Agar (VRBA) (Merck, Darmstadt, Germany) after an incubation period of 24 h at 37±0.5 ºC. Microorganisms exhibiting a proteolytic or lipolytic phenotype were determined on casein-agar medium or tributyrine-agar, respectively, after incubation at 30ºC for 48 h, as previously described [31].

In all cases, microbial counts were transformed into log $CFU \cdot g^{-1}$ white muscle before undergoing statistical analysis. All analyses were conducted in triplicate.

Statistical analysis

Data obtained from the different chemical and microbiological analyses were subjected to the ANOVA method to explore differences resulting from the effect of the icing 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 batches were considered significant for a confidence interval at the 95% level ($p < 0.05$) in all cases.

RESULTS AND DISCUSSION

Effect of U. pinnatifida on lipid hydrolysis events

A marked development of lipid hydrolysis could be observed throughout the chilling storage in all batches of this lean (lipid content: 5.0-5.5 g kg^{-1} muscle) fish species (Fig. 1). Comparison among batches revealed lower average values for fish specimens stored in ice including the alga extract. Such differences were found to be significant ($p < 0.05$) at the end of the storage time. These results allow concluding an inhibitory effect as a result of the presence of the alga extract in the icing system. Interestingly, fish specimens corresponding to IS-2 batch depicted lower average values than counterparts corresponding to the blank control.

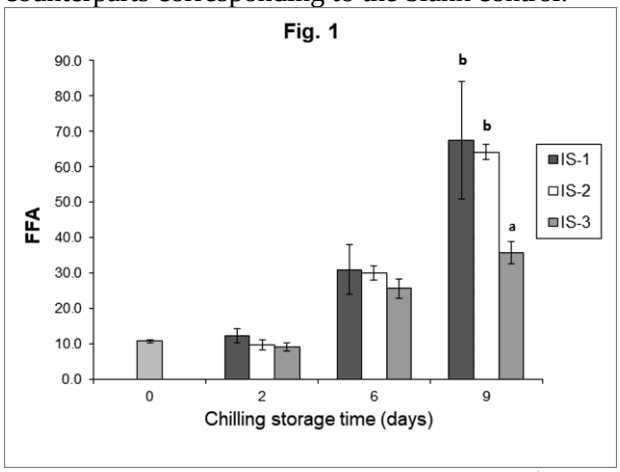

Figure 1: Evolution of free fatty acid (FFA; $g \cdot kg^{-1}$ lipids) content* on megrim muscle stored under different icing conditions**

* Average values of three replicates ($n = 3$); standard deviations are indicated by bars. Values accompanied by different letters indicate significant differences ($p < 0.05$) as a result of the icing system applied.

** Icing conditions: IS-1 (blank control), IS-2 (ethanol control) and IS-3 (alga extract treatment), as expressed in the Material and Methods section.

FFA formation during fish chilled storage has been explained as a result of both endogenous enzyme and microbial activity [1, 2]. Before the end of the microbial lag phase (*ca.* 6-9 days), FFA formation is mostly caused by endogenous enzyme activity (i.e., lipases and phospholipases); later on, microbial activity should be the predominant mechanism of generation. Current data show the highest differences at the end of the chilled storage, when the microbial activity is expected to be more intense and the inhibitory effect of the alga extract would be more likely to act.

Previous research showed an inhibitory effect of algae extracts on FFA formation in chilled fish. This applies to *Bifurcaria bifurcata* and chilled megrim (*L. whiffiagonis*) [8], and to *Fucus spiralis* and chilled hake (*M. merluccius*) [32]. Contrary, the

employment of *Gracilaria gracilis* extracts in the icing medium during the chilled storage of hake did not lead to a beneficial effect [9]. Interestingly, extracts obtained from other kinds of natural sources such as rosemary and oregano [4] or jumbo squid (*Dosidicus gigas*) skin [33] also implied inhibition of lipid hydrolysis mechanisms in stored fish.

A slight formation of peroxides could be observed in all batches throughout chilled storage (Table 1). Interestingly, values were in all cases below 5.0, this indicating a low formation of this kind of lipid oxidation compounds. Comparison among samples revealed higher average values in treated fish (i.e., IS-3 condition) in the 6-9-day period.

Effect of U. pinnatifida on lipid oxidation events

Table 1. Assessment of lipid oxidation development* in chilled megrim muscle stored under different icing systems**

Chemical index/ icing system	Chilling storage time (days)			
	0	**2**	**6**	**9**
Peroxide value				
IS-1	2.10 (0.45)	1.82 (1.02)	2.12 a (0.60)	1.65 (0.57)
IS-2		0.96 (0.64)	2.05 a (0.38)	1.73 (0.33)
IS-3		1.44 (0.31)	4.61 b (0.47)	3.54 (1.66)
Thiobarbituric acid index				
IS-1	0.16 (0.05)	0.35 (0.18)	0.40 b (0.06)	0.40 (0.14)
IS-2		0.25 (0.04)	0.23 a (0.00)	0.40 (0.10)
IS-3		0.23 (0.15)	0.27 a (0.07)	0.45 (0.09)
Fluorescence ratio				
IS-1	0.65 (0.07)	1.49 (0.16)	2.06 b (0.07)	7.53 b (1.33)
IS-2		1.61 (0.18)	1.35 a (0.13)	4.17 a (0.63)
IS-3		1.53 (0.17)	1.26 a (0.51)	3.26 a (0.94)

* Average values of three replicates (n = 3); standard deviations are indicated in brackets. Average values followed by different letters indicate significant differences ($p < 0.05$) as a result of the icing condition. Units employed: meq. active oxygen·kg^{-1} lipids (peroxide value) and mg malondialdehyde·kg^{-1} muscle (thiobarbituric acid index).

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

In global terms, a low formation of secondary lipid oxidation compounds (i.e., TBARS) was also observed throughout chilled storage, with values below 0.50 in all cases (Table 1). Lower average values could be observed in fish specimens corresponding to IS-2 and IS-3 batches for the 2-6-day period as compared to their counterparts from the blank control. However, no differences ($p > 0.05$) could be observed between both batches including ethanol in the icing system, so that a beneficial effect of the alga extract presence in the icing medium could not be inferred at this level.

The assessment of tertiary lipid oxidation development depicted a marked formation throughout storage for all batches (Table 1). For the 6-9-day period, lower average scores were determined in the IS-3 batch as compared to both controls (i.e., IS-1 and IS-2 batches). Consequently, a partial inhibition of lipid oxidation events could be concluded as a result of the presence of the alga extract in the ice system. This inhibitory effect is in agreement with its profitable polyphenol content (11.0±1.0 mg GAE·g^{-1} lyophilised alga) and antioxidant capacity (27% according to the DPPH assay). Furthermore, previous research reported on the antioxidant properties of this brown seaweed on the basis of its chemical composition and different *in-vivo* and *in-vitro* assays [15, 16]. In our study, this beneficial effect has been proven in lean fish subjected to refrigerated storage in an icing system including an extract of this alga.

Lipid oxidation has been recognised as a complex process where different kinds of molecules are produced, most of them unstable, susceptible to breakdown and to originate low-molecular weight compounds, or to react with other molecules (i.e., nucleophilic-type) present in fish muscle. In the present study, low concentrations of primary and secondary lipid oxidation compounds (i.e., peroxides and TBARS) were determined during chilled storage, while a marked increase in tertiary compounds content was observed. It could be concluded that interactions between primary and secondary lipid oxidation compounds with nucleophilic compounds present in the fish muscle was likely to occur, this leading to a notable formation of fluorescent compounds [28].

The presence of other algae extracts in the icing media has been reported to exert inhibitory effects on lipid oxidation events in fish during chilled storage. Thus, the formation of tertiary lipid oxidation compounds was partially inhibited in chilled hake by the presence of *G. gracilis* extracts [9], as well as in chilled megrim by the presence of *B. bifurcata* extracts [8]. The presence in the icing medium of extracts from other natural sources also led to increased stability against rancidity. This is the case of sardine (*Sardinella aurita*) when rosemary extract was included in the chilling medium [5] and Chilean jack mackerel (*Trachurus murphyi*) when oregano or rosemary were included in the ice systems [4].

Table 2. Evolution of aerobes, proteolytics and lipolytics counts (log CFU·g^{-1} muscle)* in chilled megrim muscle stored under different icing systems**

Bacterial group/ icing system	Chilling storage time (days)			
	0	2	6	9
Aerobes				
IS-1	2.69 (0.53)	2.78 (0.08)	4.28 b (0.19)	5.65 b (0.24)
IS-2		2.34 (0.29)	4.03 ab (0.29)	3.87 a (0.85)
IS-3		2.52 (0.24)	3.60 a (0.25)	3.65 a (0.88)
Proteolytics				
IS-1	2.36 (0.39)	2.62 (0.28)	4.50 b (0.51)	5.55 b (0.22)
IS-2		2.10 (0.17)	3.75 ab (0.72)	4.03 a (0.50)
IS-3		2.46 (0.41)	3.47 a (0.40)	4.03 a (0.56)
Lipolytics				
IS-1	2.00 (0.00)	2.00 (0.00)	3.19 (0.78)	4.57 b (0.37)
IS-2		2.00 (0.00)	3.17 (0.36)	3.38 a (0.43)
IS-3		2.30 (0.30)	2.92 (0.66)	3.16 a (0.27)

* Average values of three replicates (n = 3); standard deviations are indicated in brackets. Average values followed by different letters indicate significant differences ($p < 0.05$) as a result of the icing condition.

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

Effect of U. pinnatifida on microbial activity

A marked increase of aerobe counts was observed in all batches throughout storage (Table 2). Interestingly, fish specimens corresponding to the treated batch exhibited lower average values than their counterparts belonging to both controls at advanced storage (6-9 days). However, differences with respect to IS-2 batch were not found to be significant ($p > 0.05$).

A negligible growth of Enterobacteriaceae was observed in all batches (data not shown), with counts in all cases below 1.00, so that no significant differences ($p > 0.05$) among batches could be inferred.

The numbers of proteolytic and lipolytic bacteria increased as storage time progressed. However, marked lower average scores for both microbial groups were observed in fish specimens

corresponding to the treated batch as compared to counterparts belonging to both control batches for the 6-9-day period. Differences were found to be significant ($p < 0.05$) when compared to the blank control (6-9-day period for proteolytic counts; day 9 for lipolyic counts). However, differences were not significant ($p > 0.05$) when compared to the ethanol control (IS-2 batch). From these results it can be concluded a slight inhibitory effect of the alga extract present in the ice system on microbial activity in megrim muscle. Interestingly, previous research accounted for the antimicrobial properties of "wakame" according to different *in-vitro* assays against different kinds of pathogenic bacteria [15, 16].

The presence of natural components with antimicrobial activity is widespread in macroalgae, and a wide variety of metabolites such as terpenes, polyphenols, oligomeric pholorotannins, steroids, halogenated ketones and alkanes, fucoxanthin, polyphloroglucinol, bromophenols, etc. has been isolated and characterised [34, 35]. Previous studies have shown an inhibitory effect on microbial activity derived from the presence of algae extracts in the icing medium. These results account for *B. bifurcata* during the chilled storage of megrim [8] and *F. spiralis* when applied to chilled hake [29]. A similar inhibitory effect could be observed by employing a rosemary extract with chilled sardine [5] or a wild-thyme hydrosol extract with chilled Transcaucasian barb (*Capoeta capoeta capoeta*) [3].

CONCLUSIONS

Bioactive extracts of *U. pinnatifida* have been used to our knowledge for the first time with the aim of enhancing the quality of seafood. As a result, the inclusion of ethanolic alga extracts in the icing medium employed for fish refrigerated storage led to an inhibitory effect on lipid hydrolysis mechanisms (FFA formation), and a partial inhibition of lipid oxidation events (fluorescent compounds) and microbial activity (aerobes, proteolytic and lipolytic bacteria).

The results presented in this work open the way to the potential application in food systems of the alga *U. pinnatifida* as a source of bioactive extracts, and specifically for chilled fish on-board and in-land storage applications. The proposed novel icing system can be considered of interest due to the simple methodology employed and the resulting protective effects observed on fish quality. Further research aimed at optimising the experimental conditions (namely, extract concentration) for different kinds and types of fish species ought to be developed.

Acknowledgements. *The authors thank Fernando G. Della Torre (Ph. D.) for kindly selecting the fresh alga. This work was supported by the 2013 Cooperation Program "CONICET (Argentine)-CSIC (Spain)" and by the Project PIE 2013-70E001 (CSIC, Spain).*

REFERENCES

1. I. Ashie, J. Smith, B. Simpson, *Crit. Rev. Food Sci. Nutrit.*, **36**, 87 (1996).
2. C. Campos, M. Gliemmo, S. P. Aubourg, J. Barros-Velázquez, in: Novel Technologies in Food Science, A. McElhatton, P. Amaral Sobral (eds), chapter 13, Springer, New York, USA, 2012, p. 299.
3. N. Oral, M. Gülmez, L. Vatansever, A. Güven, *J. Food Protect.*, **71**, 218 (2008).
4. V. Quitral, MªL. Donoso, J. Ortiz, MªV. Herrera, H. Araya, S. P. Aubourg, *LWT-Food Sci. Technol.*, **42**, 1450 (2009).
5. G. Özyurt, E. Kuley, E. Balikçi, Ç. Kaçar, S. Gökdogan, M. Etyemez, F. Özogul, *Food Bioproc. Technol.*, **5**, 2777 (2012).
6. K. Sallam, *Food Cont.*, **18**, 566 (2007).
7. B. García-Soto, I. Fernández-No, J. Barros-Velázquez, S. P. Aubourg, *Int. J. Refrig.*, **40**, 390 (2014).
8. J. M. Miranda, J. Ortiz, J. Barros-Velázquez, S. P. Aubourg, *Food Bioproc. Technol*., **9**, 387 (2016).
9. R. G. Barbosa, M. Trigo, G. Dovale, A. Rodríguez, S. P. Aubourg, *Bulg. Chem. Comm*., **50**, 118 (2018).
10. Mª E. Díaz-Rubio, J. Pérez-Jiménez, F. Saura-Calixto, *Int. J. Food Sci. Nutrit.*, **60**, 23 (2009).
11. L. Paiva, E. Lima, R. Ferreira Patarra, A. Neto, J. Baptista, *Food Chem.*, **164**, 128 (2014).
12. S. Halldorsdóttir, H. Sveinsdóttir, A. Gudmundsdóttir, G. Thorkelsson, H. Kristinsson, *J. Funct. Foods*, **9**, 10 (2014).
13. E. Sandsdalen, T. Haug, K. Stensvag, O. Styrvold, *World J. Microb. Biotechnol.*, **19**, 777 (2003).
14. F. G. Dellatorre, R. Amoroso, J. Saravia, J. Orensanz, *Aquat. Invas.*, **9**, 467 (2014).
15. J. K. Patra, S. W. Lee, J. G. Park, J. H. Baek, *J. Food Biochem*., **41**, e12278 (2017).
16. L. Wang, Y. J. Park, Y. J. Jeon, B. M. Ryu, *Aquacult*., **495**, 873 (2018).
17. S. Kumar, D. Sahoo, I. Levine, *Algal Res*., **9**, 117 (2015).
18. S. M. Rafiquzzaman, E. Y. Kim, J. M. Lee, M. Mohibbullah, M. Badrul Alam, I. Soo Moon, J. M. Kim, I. S. Kong, *Food Res. Internat*., **77**, 118 (2015).
19. P. Prabhasankar, P. Ganesan, N. Bhaskar, A. Hirose, N. Stephen, L. Gowda, M. Hosokawa, K. Miyashita, *Food Chem*., **115**, 501 (2009).
20. D. M. Kim, K. H. Kim, H. S. Yook, *J. Korean Soc. Food Sci. Nutr*., **41**, 103 (2012).
21. D. M. Kim, K. H. Kim, Y. S. Yun, J. H. Kim, J. W. Lee, H. S. Yook, *J. Korean Soc. Food Sci. Nutrit*., **40**, 1460 (2011).
22. S. Kim, S. Woo, H. Yun, S. Yum, E. Choi, J. R. Do, J. H. Jo, D. Kim, S. Lee, T. K. Lee, *Food Sci. Biotechnol*., **14**, 798 (2005).

23. G. Yen, H. Y. Chen, *J. Agric. Food Chem*., **43**, 27 (1995).
24. E. Bligh, W. Dyer, *Can. J. Biochem. Physiol.*, **37**, 911 (1959).
25. R. Lowry, I. Tinsley, *J. Amer. Oil Chem. Soc.*, **53**, 470 (1976).
26. R. Chapman, J. McKay, *J. Am. Oil Chem. Soc.*, **26**, 360 (1949).
27. W. Vyncke, *Fette Seifen Anstrichm.*, **72**, 1084 (1970).
28. S. P. Aubourg, *J. Am. Oil Chem. Soc*., **76**, 409 (1999).
29. B. Ben-Gigirey, J. Vieites Baptista de Sousa, T. Villa, J. Barros-Velázquez, *J. Food Protect*. **61**, 608 (1998).
30. B. Ben-Gigirey, J. Vieites Baptista de Sousa, T. Villa, J. Barros-Velázquez, *J. Food Protect*. **62**, 933 (1999).
31. B. Ben-Gigirey, J. Vieites Baptista de Sousa, T. Villa, J. Barros-Velázquez, *Int. J. Food Microb.*, **57**, 19 (2000).
32. J. Barros-Velázquez, J. M. Miranda, J. M. Ezquerra-Brauer, S. P. Aubourg, *Int. J. Food Sci. Technol.*, **51**, 2081 (2016).
33. J. M. Ezquerra-Brauer, M. Trigo, W. Torres-Arreola, S. P. Aubourg, *Bulg. Chem. Comm*., **50**, 131 (2018).
34. S. Gupta, N. Abu-Ghannam, *Trends Food Sci. Technol.*, **22**, 315 (2011).
35. J. Fleurence, M. Morançais, J. Dumay, P. Decottignies, V. Turpin, M. Munier, N. García-Bueno, P. Jaouen, *Trends Food Sci. Technol.*, **27**, 57 (2012).