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Development of functional bio-based seaweed (*Himanthalia elongata* and *Palmaria palmata*) edible films for extending the shelflife of fresh fish burgers

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ABSTRACT

Seaweeds (*Himanthalia elongata* and *Palmaria palmata*) and seaweed extracts were used for formulation of active edible films. Films formulated with *H. elongata* showed higher total phenols and antioxidant capacity than the films formulated with *P. palmate* and significant ($p \leq 0.05$) higher than those formulated with seaweeds extracts regardless of the specie used.

The use of the edible films enriched with seaweeds in the fish burgers controlled significantly pH and water activity changes over storage and reduced the microbial growth, especially in the case of edible films formulated with *H. elongata* compared to control. In addition, a significant ($p \leq 0.05$) reduction of lipid oxidation and enhancement of antioxidant capacity of trout burgers over storage were observed. The use of seaweeds incorporated in edible films seems to be a feasible strategy for increasing the shelf life of fish burgers, products prone to rapid oxidative processes and spoilage.

1. Introduction

Nowadays, most of the food packaging is made of plastic, being the global production in 2015 of more than 300 million metric tons (Worm, Lotze, Jubinville, Wilcox, & Jambeck, 2017). Packaging is the largest sector currently in Europe; namely, 40% of plastic is allocated to packaging (Europe, 2016). Plastic of non-biodegradability nature has led to a serious sustainability issue. The development of edible films and coatings is an environmentally friendly technology that would permit a reduction in the impact and disposal costs associated with synthetic polymeric films (Silva-Weiss, Ihl, Sobral, Gómez-Guillén, & Bifani, 2013). Edible bio-based films are promising packaging systems due to non-polluting nature and although currently edible films do not entirely replace traditional packaging, due to handling and hygiene reasons, they can reduce the use of conventional packaging (Cordeiro de Azeredo, 2012). Edible films and coatings have been extensively studied in recent years due to their unique properties and advantages over more traditional conservation techniques. Edible films and coatings improve shelf life and food quality, by providing a protective barrier against physical and mechanical damage, and by creating a controlled atmosphere and acting as a semipermeable barrier for gases, vapour, and water (Peltzer, Salvay, Delgado, & Wagner, 2016). Thus, the use of edible films allows extending the shelf life of many food products. In addition, edible films are very good carriers for the delivery of bioactive compounds through gradual liberation over storage (Campos, Gerschenson, & Flores, 2011). Edible films have been successfully combined, within a hurdle strategy, with other technologies as for example High Pressure Processing (Albertos, Rico et al., 2015). The application of edible films could also be an option for the design of products with improved properties; such as reducing fat uptake in deep fat fried products (Kurek, Ščetar, & Galić, 2017). Formulation of these films is based on polymers such as polysaccharides (starch, chitosan, cellulose, pectin, alginate, *i.e.)* or proteins (gelatin, whey protein, casei, *i.e.)* mainly (Campos et al., 2011; Hassan, Chatha, Hussain, Zia, & Akhtar, 2018; Salgado, Ortiz, Musso, Di Giorgio, & Mauri, 2015; Sánchez-González, Vargas, González-Martínez, Chiralt, & Cháfer, 2011).

Seaweeds have been consumed in Asian and Pacific countries since ancient times and gained popularity in Europe and America. Farmed aquatic plants, above all seaweed industry, have been expanded at 8% in the past decade, up from 6.2% in the previous decade, with output more than doubling in this period (FAO, 2016). This increasing interest is probably due to the fact that seaweeds are rich in bioactive compounds, such as dietary fibre, high-quality protein, abundant minerals, vitamins, presence of unsaturated essential fatty acids, polyphenols, carotenoids, tocopherol, *etc.*(Cofrades, López-López, Ruiz-Capillas, Triki, & Jiménez-Colmenero, 2011; Ferraces-Casais, Lage-Yusty, de Quirós, & López-Hernández, 2012; Jimnez-Escrig, Gmez-Ordez, &

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Ruprez, 2011; Kadam, Álvarez, Tiwari, & O'Donnell, 2015; Mabeau & Fleurence, 1993). Brown and red seaweeds are also considered as food or food ingredients in European countries (Regulation, 1997 (EC) 258/97). Seaweeds have been reported to act as antioxidant and antimicrobial ingredients by different authors (Belda et al., 2016; Cox & Abu-Ghannam, 2013; Eom, Kim, & Kim, 2012; Ferraces-Casais et al., 2012; Gupta, Cox, Rajauria, Jaiswal, & Abu-Ghannam, 2012; Yuan, Bone, & Carrington, 2005).

The use of edible seaweeds or seaweed-derived extracts seems to be an interesting strategy to develop natural functional food products. Previous works have investigated the incorporation of seaweed extracts in edible films (Balti et al., 2017; Blanco-Pascual, Montero, & Gmez-Guilln, 2014; Haddar et al., 2012; Kadam, Pankaj, Tiwari, Cullen, & O'Donnell, 2015; Rattaya, Benjakul, & Prodpran, 2009); however fewer works have been applied these films in a food product and evaluated the effects on its shelf life (Yang, Lee, Lee, & Song, 2017). Based on our knowledge, no information on the use of edible films containing seaweeds in fish processing products has been reported to date.

Rainbow trout (*Oncorhynchus mykiss*), as most fish, contains a high proportion of polyunsaturated fatty acids (PUFAs), making it highly susceptible to oxidation, development of unpleasant off-flavours and microbial spoilage (Baron, Kjærsgård, Jessen, & Jacobsen, 2007). Therefore, the aim of this work was to develop an edible film formulated with seaweeds with ability to extend the shelf life of fish burgers over storage.

2. Materials

2.1. Chemicals

All the chemicals used in the formulation of edible films were food grade (Panreac Química, Barcelona, Spain). Other reagents were purchased to Sigma-Aldrich (Sigma Aldrich Chemical Co Steinheim, Germany). All the solvents were HPLC grade (Lab-Scan, Dublin, Ireland). Buffered peptone water was obtained from AES (Cambourg, France) and microbial culture media (PCA, MRS and VRBL) from Biolife (Milan, Italy).

2.2. Preparation of seaweeds extracts

Himanthalia elongata and *Palmaria palmata* supplied by Porto Muiños (Cerceda, A Coruña, Spain) as dry powder (< 2% water content) were dissolved in distilled water (5% w/v) for 1 h stirring at room temperature. The suspension homogenized was after centrifugate (4,000*xg* for 15 min) and supernatant was separated and filtered obtaining the fraction A. After pellet was dissolved again on distilled water at 95 °C with stirring for 1 h and it was cooled at room temperature before new centrifugation (4,000*xg* for 15 min) to obtain the second fraction named B. Both fraction A and B were stored at −20 °C for further use.

2.3. Preparation of edible films enriched with seaweeds and seaweed extracts incorporation

Edible films were prepared dissolving 1.5% of chitosan into the aqueous solution of 1% acetic acid. The suspension was stirred at 40 °C for 2 h using an enzymatic digester (GDE, Velp Scientifica, Italy) to obtain a homogenous solution. Afterwards, a volume of 0.5 g glycerol per g of biopolymer was added as plasticiser and stirred for 2 h to achieve complete dispersion of the mixture. Seaweeds and seaweed extracts were incorporated in this step. *H. elongata* and *P. Palmata* were added directly at 1% in edible films (FH, FP) and edible films with *H. elongata* extract (FHE) and *P. palmata* extract (FPE) were prepared by adding chitosan and seaweed extract at a 2:1:1 (v:v:v) ratio (chitosan: seaweed fraction A: seaweed fraction B). Edible films without extracts were prepared as control.

The mixture (20 ml) were cast onto 90 mm-diameter Petri dishes, and dried at 42 °C in an air-forced incubator (Biosan ES-20, Biogen Científica SL, Madrid, Spain) for 15 h and subsequently cooled at room temperature for 24 h. Prior to analyses, the films were removed out from glasses plates and stored in desiccators over a saturated solution of KBr (58% relative humidity).

3. Methods

3.1. Characterization of extracts and formulate edible seaweeds

3.1.1. Antioxidant capacity of seaweeds

Extracts were used for evaluation of total phenols (TP), TEAC (Trolox Equivalent Antioxidant Capacity), ORAC (Oxygen Radical Absorbance Capacity) and FRAP (Ferric Ion Reducing Antioxidant Power) were determined in all the extracts.

TP were measured using the Folin-Ciocalteu method (Slinkard & Singleton, 1977) and results were expressed as mg gallic acid/g sample. TEAC was carried out according Re et al. (1999) and results were expressed as mmol Trolox equivalents (TE)/g sample. ORAC was based on the method described by Ou, Hampsch-Woodill, and Prior (2001)) and results were expressed as mmol Trolox equivalent (TE)/g sample. FRAP was determined according to the procedure described by Pereira et al. (2008) and results were expressed as μmol Fe equivalent/g sample.

3.1.2. Antioxidant capacity of edible films formulated with seaweeds or seaweed extracts

A piece of edible film of 200 mg was placed in a polypropylene tube containing 30 mL of methanol. Mixture was stirred at 3000 x g for 90 min at room temperature and centrifugate at 3214 *x g* for 10 min, the supernatant was collected and stored for antioxidant capacity and total phenol determinations following the methods described in the Section 3.1.

3.1.3. Edible films thermal properties of edible films formulated with seaweeds or seaweed extracts

The films were conditioned in a desiccator containing silica gel for further studies such as film characterization using different analytical methods and for coating fresh fish burgers:

Fourier-transform infrared spectroscopy (FTIR spectroscopy).

3.1.3.1. Pieces of film of 2 cm-diameter were sandwiched between two KBr disks. The Fourier transform infrared (FT-IR) spectra of films were recorded in an IR spectrometer (Nicolet iS10,Thermo Fisher Scientific, Waltham M.A, USA), provided with attenuated total reflectance accessory (ATR) with a SeZn glass (smartiTR, Thermo Fisher Scientific, Waltham M.A, USA). Samples were recorded from wave number 4000-600 cm^{-1}. Signal averages were obtained from 32 scans at a resolution of 4 cm^{-1}. The spectra obtained were used to explain interaction between seaweeds and chitosan.

3.1.3.2. Thermogravimetric analysis (TGA). Thermal stability of the films was analyzed by thermogravimetric analyzer (SDT Q600, TA Instruments, New Castle DE, USA). Film pieces (10 mg) were placed in aluminum pans, sealed and scanned over the range of 50–150 °C in nitrogen atmosphere with heating rate of 20 °C/ min.

3.1.3.3. Differential scanning calorimetry (DSC). Thermal properties of the films were determined using a differential scanning calorimetry (DSC) (Q2000, TA Instruments, New Castle DE, USA). Films (10 mg) were placed in aluminum pans with hole. The nitrogen flux was 50 mL/min. The first ramp was from 30 °C to 200 °C with a heating rate of 20 °C /min, followed by a 5 min isotherm. After this, films were cooled down to 30 °C at a rate of 20 °C/min followed by a 5 min isotherm in nitrogen atmosphere. Finally, films were heated up to 250 °C at a rate of 40 °C/min to determinate the glass transition temperatures (Tg), fusion temperature and fusion enthalpy. The empty aluminum pan was used

as a reference. The analyses were replicated.

3.2. Effect of edible films on fish burgers shelf life

3.2.1. Fish burger preparation

Gutted aquaculture rainbow trout (*Oncorhynchus mykiss*) were provided by aquaculture local farm IPEASA (Villa de Fuentidueña, Segovia, Spain). Fillets were manually skinned and minced using a blender with a 7 mm exit pore (Lacor 69067, Guipúzcoa, Spain). Burgers (50 g) were prepared manually with a round-shaped mould. Burger were coated aseptically at room using edible film formulated with *H. elongata* seaweed (FH), edible film *P. palmata* seaweed (FP), edible film *H. elongata* seaweed extract (FHE) and edible film *P. palmata* extract (FPE). Fresh fish burgers with edible coating without seaweed in the formula was used as control. All samples were placed in trays cover with aluminium foil and stored at 4 °C until analysis.

All samples were kept at 4 °C storage for 7 days, and 4 points were chosen at day 0, 2, 5 and 7 days to evaluate the influence of edible films application on quality characteristics. All analyses were performed in two different batches and in triplicate.

3.2.2. Physicochemical properties

Physicochemical properties analyzed included pH, water activity, Thiobarbituric acid reactive substances and colour.

For pH 10 g-sample of fish burger was homogenised in 100 mL of distilled water and the mixture filtered. The pH of the filtrate was measured at room temperature (pH-meter model 507, CRISON, Barcelona, Spain).

Water activity (aw) was measured with an Aqualab 4TE water activity meter (Decagon Devices Inc, Pullman, WA, USA) for sample of fish burger.

Colour parameters (lightness L*, redness a* and yellowness b*) were measured using a spectrophotometer (Minolta CM-2002, Osaka, Japan). The illuminant was D65 and10° standard observers. Measurements were taken on burgers without edible films at eight different points in order to homogeneously screen the surface.

Thiobarbituric acid reactive substances (TBARS) was analysed in samples following the method described by Vyncke (1975). Results were expressed as μmol malondialdehyde (MDA)/g of muscle.

3.2.3. Microbiological analysis

Standard methods were used to conduct microbial analysis. For microbial analysis 25 g of fish burger were aseptically transferred into bags (Microgen, Surrey, United Kingdom) with 90 mL of sterile Buffered Peptone Water and homogenised with a pulsifier for 90 s (Pul 100E, Microgen, Surrey, United Kingdom). For each sample, appropriate serial decimal dilutions were prepared in Buffered Peptone Water solution (1 g per L) for the following microorganism counts: Total aerobic mesophilic were determined using Tryptic Glucose Yeast Agar (PCA) after incubation at 30 °C for 72 h. Total psychrotrophic bacteria on PCA spread plates, were determined after incubation at 4 °C for 10 days.

3.2.4. Antioxidant capacity

Antioxidant activity of burgers coated with edible films were analysed. One gram of burger was extracted following the method of Sánchez-Alonso et al. (2008). ORAC determination on flesh fish was performed in triplicate, the method used was the one described in Section 3.1.1.

3.3. Statistical analysis

Data were analysed by one-way ANOVA. Fisher LSD (Least Significant Difference) test was applied for determining group differences at 95% significance level. Statgraphics Centurion XVI was used for carrying out the statistical analysis.

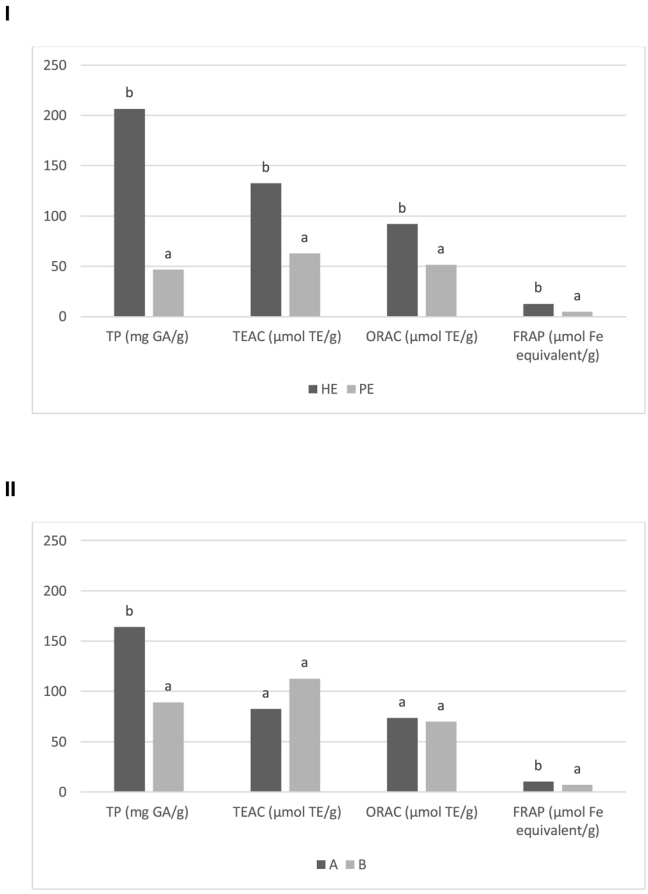


Fig. 1. Total phenols and antioxidant capacity (TEAC, ORAC, FRAP) of *Himanthalia elongate* (HE) and *Palmaria palmate* (PE) *extracts* (I) and total phenols and antioxidant capacity (TEAC, ORAC, FRAP) for fraction A and fraction B. For each marker different letter indicate significantly different ($p < 0.05$).

4. Results and discussion

4.1. Antioxidant capacity of seaweed extracts and edible films formulated with seaweeds and seaweed extracts

First all the seaweeds were characterized from antioxidant point of view. *H. elongata* extract (HE) showed a significantly higher total polyphenolic content (206.69 mg GAE/g) than *P. palmata* extract (PE) (46.72 mg GAE/g). The higher total phenolic content of HE may possibly be responsible of the higher antioxidant activity observed through of *in vitro* assays such as TEAC, ORAC and FRAP (Fig. 1I). Significant correlation ($p \leq 0.05$, data not shown) between total phenolic content and the antioxidant activity was observed, and this has also been reported in previous studies (Rajauria, Foley, & Abu-Ghannam, 2016; Wang, Marcone, Barbut, & Lim, 2012). Seaweed phlorotannins have been suggested to scavenge free radicals, namely, superoxide, peroxyl and nitric radicals, and chelate ferrous ions according with Roohinejad et al. (2017). Ferraces-Casais et al. (2012) also found that *H. elongata* had significantly higher antioxidant activity than *P. palmata*. *H. elongata* also presented higher polyphenol and vitamin C content. Similarly, results were reported by Cofrades et al. (2010) who found that *H. elongata* displayed high total phenolic content and antioxidant activity in comparison with other seaweeds studied. Among the phenolics compounds, hydroxybenzaldehyde, phloroglucinol, kaempferol, cirsimaritin, gallic acid 4-O-glucoside, carnosic acid and gallic acid were identified as the main responsible of the activity of *H. elongata* with potential antioxidant capacity (Rajauria et al., 2016).

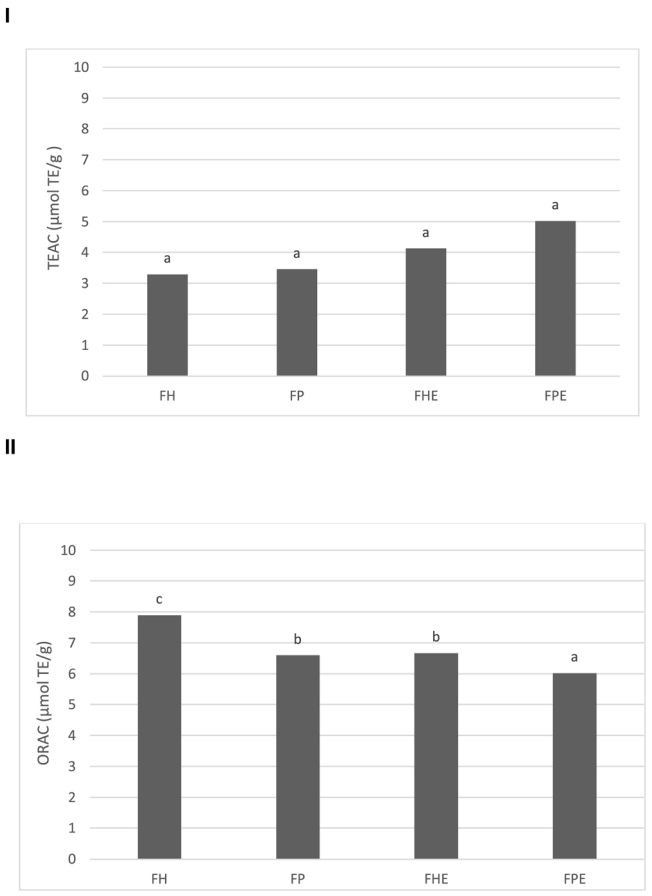


Fig. 2. Antioxidant capacity TEAC (I) and ORAC (II) of edible films formulated with seaweeds (FH, FP) and seaweeds extracts (FHE, FPE). Different letter are significantly different ($p < 0.05$).

Probably the structural complexity of the *P. palmata* cell wall (Deniaud, Quemener, Fleurence, & Lahaye, 2003) could be an obstacle for the efficient extraction of the intracellular bioactive constituents in the *P. palmata* which could contribute to the lower activity observed.

This extraction was performed with water two-steps (with or without temperature). Seaweed polyphenols were isolated using aqueous or polar solvents such as acetone or methanol (Kadam, Álvarez et al., 2015). In the first extraction (Fraction A), more total phenolic content and high FRAP were found in comparison with the repeated hot aqueous extraction (Fraction B) (Fig. 1II). Although, higher temperature corresponded with greater total phenols content in *H. elongata* polyphenols extraction (Belda et al., 2016).

The antioxidant activity also was evaluated in the edible films formulated. Results showed higher antioxidant capacity (TEAC and ORAC) was found in edible films with seaweeds (FH, FP) and seaweed extract (FHE, FPE) incorporation (Fig. 2). Edible films formulated with *H. elongata* (FH) showed the highest activity in ORAC. These results were in agreement with the values for seaweeds described in the previous section. Red seaweeds such as *P. palmata* (P) contain lower level of phenols than brown seaweeds, such as *H. elongata* (H) (Mabeau & Fleurence, 1993). Furthermore, antioxidant activity may arise not only from polyphenols, but sulfated polysaccharides, which are reported antioxidant compounds present in seaweeds (Costa, Fidelis, Rocha, & Costa, 2014). Antioxidant activity of seaweed polysaccharide components may depend on various factors, such as sulfation level, molecular weight and sugar residue composition (Jiménez-Escrig et al., 2011). *H. elongata* is a brown seaweed which synthesises polysaccharides rich in sulphated a-L-fucose, whereas *P. palmata* (P) is a red seaweed rich in galactose (Costa et al., 2014). The antioxidant fucoid, isolated from brown seaweed *Sargassum polycystum*, demonstrated higher antioxidant capacity than L-ascorbic acid (Palanisamy, Vinosha, Marudhupandi, Rajasekar, & Prabhu, 2017). Formulated edible formulated using extracts (FHE, FPE) instead of seaweeds (FH, FP) did not provide any advantage, according to results (TEAC, ORAC) obtained.

4.2. Thermal properties edible films

Since all the burger needs to be processed using temperature. Thermal behaviour of edible films was required to predict the effect on the final product.

4.2.1. Fourier-transform infrared spectroscopy (FTIR spectroscopy)

FTIR spectrum of edible films with seaweeds (FH, FP) and seaweeds extracts (FHE, FPE) exhibited the characteristic absorption bands of the

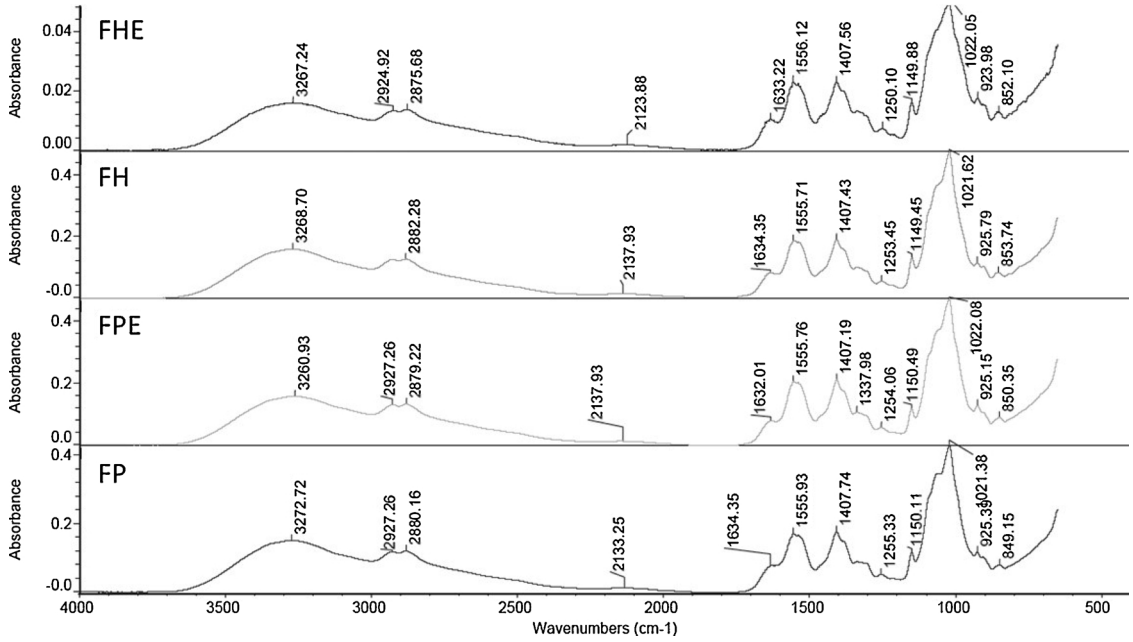


Fig. 3. Fourier-transform infrared spectroscopy (FT-IR spectrum) of edible films formulated with seaweeds (FH, FP) and seaweeds extracts (FHE, FPE).

biopolymer used (chitosan) (Fig. 3). Absorptions peaks of chitosan were located at 1633.57 cm^{-1}, related to ^{CO}= (amide I), at 1555.76 cm^{-1}, attributed to ^{NH}– stretching (amide II) and at 1337.56 cm^{-1}, assigned to ^{C}–N group (amide III). Furthermore, the absorption bands associated to OH and NH (3.600–3.400 cm^{-1}) correspond to alcohols, amines and amides also appeared. The absorption band located at 1406.29 cm^{-1} is attributed to carboxylate groups, and the absorption peak at 2927.41 cm^{-1} is typical of ^{C}–H vibration. The peaks between 896.73 and 1154.19 cm^{-1} correspond to saccharide structure of chitosan. The broad peak at 1080.91 cm^{-1} indicates ^{C}–O stretching vibration (Leceta et al., 2015).

The presence of seaweed extracts in the chitosan edible films was not clearly reflected on the FT-IT spectra. Typical absorption bands at 1200–970 cm^{-1} were detected in edible films, which contained brown seaweeds extracts. Those bands corresponded to C—C and C–O pyranoid ring stretching and C–O glycosidic bond common to all seaweed polysaccharide standards (Gómez-Ordóñez & Rupérez, 2011). This lack on the spectra can be explained by the similarity between the functional groups of chitosan and seaweeds. The majority of the absorptions bands were overlapped in this range (1200–970 cm^{-1}).

4.2.2. Thermogravimetric analysis (TGA)

TGA allows investigate structural changes due to temperature increase. The TGA of edible films with seaweeds (FH, FP, FHE, and FPE) showed breakdown in two stages. The initial weight loss (50–125 °C) was attributed to water (free and bound) and acetic acid evaporation (Moura et al., 2015).

The second weight loss corresponded to chitosan and seaweed degradation (Table 1) due to thermal and oxidative breakdown. Films showed a degradation temperature range of 282.76 °C to 270.58 °C. Previous studies have shown higher temperatures for chitosan degradation in films (290 °C) (Leceta, Peñalba, Arana, Guerrero, & De La Caba, 2015; Peniche-Covas, Argelles-Monal, & San Romn, 1993). This main stage is associated to complex degradation processes, including depolymerisation and decomposition of polymeric units (Peniche-Covas et al., 1993).

Edible films with seaweed (FH, FP, FHE) exhibited another degradation step above 800 °C (Table 1), which is not observed in films formed with chitosan only (Leceta et al., 2015; Peniche-Covas et al., 1993). In conclusion, the addition of seaweeds enhanced the thermal properties of the films, with the exception of those containing *P. Palmata* extract (FPE). This could be due to polymerization of *P. Palmata* seaweed compounds such as polysaccharides in FP films, not present in the FPE films, increasing FP film stability.

4.2.3. Differential scanning calorimetry (DSC)

DSC analyses showed the two major transitions in edible films with seaweeds (FH, FP) and seaweed extracts (FHE, FPE) (Table 2). The first weight loss phase (water and acetic acid) started around 50 °C and finished around 125 °C. It was relative to the evaporation of water and residual acetic acid (Moura et al., 2015). Edible films with seaweed presented a range of endothermic peak specific energy values (170.9–197.1 J/g) higher than that reported for chitosan-only films, below 100 J/g (Gómez-Estaca, Gómez-Guillén, Fernández-Martín, & Montero, 2011; Jridi et al., 2014). Thus, seaweed addition to edible films increased the water absorption capacity and improved the interactions between water and polymer. The glass transition temperature (TG) of chitosan film was observed at a range of 154.2 °C–157.4 °C, which is in agreement with those reported by Dong, Ruan, Wang, Zhao, and Bi (2004)). The addition of seaweeds (FH, FP) and their extracts (FHE, FPE) did not modify TG of chitosan films. TG is an important criterion for the miscibility of components (Fakhreddin Hosseini, Rezaei, Zandi, & Ghavi, 2013), increasing film mechanical properties. It can be concluded that the incorporation of seaweeds (FH, FP, FHE, FPE) did not affect negatively to chitosan films.

Table 1
. Thermogravimetric analysis (TGA) of edible films formulated with *Himanthalia elongate* (FH) and *Palmaria palmate* (FP) seaweed and with extracts of *Himanthalia elongate* (FHE) and *Palmaria palmate* (FPE).

Edible film	Chitosan degradation temperature (ºC)	Seaweed degradation temperature (ºC)
FH	270.58	854.48
FP	271.78	890.80
FHE	282.76	879.81
FPE	272.96	ND

For each marker different letter indicate significantly different ($p < 0.05$).

Table 2
Onset and fusion temperature and enthalpy obtained in the first heating and glass transition temperature obtained in the second heating for edible films formulated with *Himanthalia elongate* (FH) and *Palmaria palmate* (FP) seaweed and with extracts of *Himanthalia elongate* (FHE) and *Palmaria palmate* (FPE).

Edible film	Onset T(ºC) First heating ramp	Fusion T (ºC) First heating ramp	AH (J/g) First heating ramp	Tg (ºC) Second heating ramp
FH	68.3	123.3	177.9	156.6
FP	54.4	124.7	197.1	157.4
FHE	64.2	124.5	170.9	154.2
FPE	65.4	122.2	180.2	156.6

4.3. Effect of edible films on fish burger shelf life

4.3.1. Physicochemical properties

The initial pH value (6.56 ± 0.03) of rainbow trout burgers was in agreement with that observed by Chytiri, Chouliara, Savvaidis, and Kontominas (2004)). Control samples showed the highest pH value over storage (Table 3). This increment in pH could be due to alkaline compound degradation, which are formed from protein and nucleotide decomposition from muscle during the post-mortem period (Mexis, Chouliara, & Kontominas, 2009). The pH of fresh fillets is almost neutral and this rise in pH affected negatively sensorial characteristics such as odor, color and texture.

Water activity plays an important role in the fish spoilage and the growth of microorganisms. The a_w values did not show a clear trend during time **(**Table 3**).** The use of seaweed films produced the lowest a_w values at the end of storage, regardless of the species used (*H. elongata* and *P. palmata*). This decrease in the a_w can be related to minor microbial counts in these samples (FH and FP). In contrast, the incorporation of extract (FHE and FPE) affected negatively to the films, increasing a_w of these samples.

Colour was evaluated and it was observed than edible films did not produced colour modifications in the final product. This fact was beneficial due to colour changes, which probably may reduce the acceptability of products.

Seaweeds (FH, FP) and extract (FHE) films samples had lower a* value than control (Table 4). Redness could provide information regarding oxidation changes (Albertos, Jaime, María Diez, González-Arnaiz, & Rico, 2015). However, in this case, this decrease in redness is caused by the migration of seaweeds films components from the film to the fish. The addition of *H. elongata* in meat products reduced the redness of meat according with results published by Cofrades et al. (2011) and Cox and Abu-Ghannam (2013). This modification can be attributed to different pigments, which are presented in *H. elongata* such as chlorophylls, phycophine (brown) and xanthophylls (yellow). Similar results were obtained with *P. palmata*, which contained a variety of carotenoids pigments such as lutein (yellow) and α and β-carotene. Edible films with *P. palmata* extract (FPE) did not exert colour modification due to the lipophilic nature of carotenoids, not being present in the extracts (Dawson, 2007). Edible film with seaweeds (FP,

Table 3
pH and water activity (aw) for fresh fish burgers coated with edible films formulated with *Himanthalia elongate* (FH) and *Palmaria palmate* (FP) seaweed and with extracts of *Himanthalia elongate* (FHE) and *Palmaria palmate* (FPE) and stored at 4 °C for 7 days. Values (mean ± standard deviation, n = 3) followed by different uppercase letter in same column and lowercase letter in same file are significantly different ($p < 0.05$).

		Storage (days)			
	Edible films	0	2	5	7
pH	**Control**	$^{A}6.56 \pm 0.04_{a}$	$^{A}6.61 \pm 0.04_{b}$	$^{A}6.55 \pm 0.04_{b}$	$^{A}6.54 \pm 0.04_{b}$
	FH	$^{B}6.56 \pm 0.04_{a}$	$^{B}6.52 \pm 0.03_{a}$	$^{A}6.47 \pm 0.03_{a}$	$^{A}6.47 \pm 0.04_{ab}$
	FP	$^{B}6.56 \pm 0.04_{a}$	$^{B}6.53 \pm 0.03_{a}$	$^{A}6.46 \pm 0.04_{a}$	$^{A}6.43 \pm 0.04_{a}$
	FHE	$^{B}6.56 \pm 0.04_{a}$	$^{A}6.52 \pm 0.03_{a}$	$^{A}6.49 \pm 0.04_{a}$	$^{A}6.51 \pm 0.02_{b}$
	FPE	$^{B}6.56 \pm 0.04_{a}$	$^{AB}6.51 \pm 0.04_{a}$	$^{B}6.49 \pm 0.04_{a}$	$^{AB}6.51 \pm 0.03_{b}$
aw	**Control**	$^{A}0.988 \pm 0.0032_{a}$	$^{A}0.987 \pm 0.0004_{bc}$	$^{A}0.989 \pm 0.0033_{ab}$	$^{A}0.985 \pm 0.0037_{b}$
	FH	$^{B}0.988 \pm 0.0032_{a}$	$^{AB}0.986 \pm 0.0007_{ab}$	$^{AB}0.987 \pm 0.0009_{ab}$	$^{A}0.982 \pm 0.0001_{ab}$
	FP	$^{C}0.988 \pm 0.0032_{a}$	$^{AB}0.983 \pm 0.0005_{a}$	$^{BC}0.986 \pm 0.0004_{ab}$	$^{A}0.980 \pm 0.0008_{a}$
	FHE	$^{A}0.988 \pm 0.0032_{a}$	$^{A}0.986 \pm 0.0002_{bc}$	$^{A}0.986 \pm 0.0002_{a}$	$^{A}0.985 \pm 0.0002_{b}$
	FPE	$^{A}0.988 \pm 0.0032_{a}$	$^{A}0.989 \pm 0.0001_{c}$	$^{A}0.987 \pm 0.0013_{ab}$	$^{A}0.9858 \pm 0.0025_{b}$

Table 4
Colorimeter parameters for fresh fish burgers coated with edible films formulated with *Himanthalia elongate* (FH) and *Palmaria palmate* (FP) seaweed and with extracts of *Himanthalia elongate* (FHE) and *Palmaria palmate* (FPE) and stored at 4 °C for 7 days. Values (mean ± standard deviation, n = 3) followed by different uppercase letter in same column and lowercase letter in same file are significantly different ($p < 0.05$).

Edible coating	L*	a*	b*	Hue	Chroma	Colour change
Control	$47.63_{a,A}$	$9.89_{b,C}$	$17.33_{a,B}$	$1.81_{ab,AB}$	$20.02_{b,C}$	$6.33_{a,A}$
FH	$49.41_{a,B}$	$7.59_{a,A}$	$15.65_{a,A}$	$2.05_{b,C}$	$17.55_{a,A}$	$6.98_{a,A}$
FP	$47.87_{a,A}$	$7.87_{a,A}$	$16.13_{a,C}$	$2.33_{c,C}$	$17.92_{a,A}$	$6.66_{a,A}$
FHE	$47.93_{a,A}$	$8.30_{a,B}$	$16.20_{a,C}$	$1.96_{ab,B}$	$18.22_{ab,B}$	$6.10_{a,A}$
FPE	$48.8_{a,AB}$	$8.92_{ab,B}$	$15.68_{a,A}$	$1.75_{b,A}$	$18.07_{ab,B}$	$7.17_{a,B}$

FH) modified Hue and Chroma, respectively in comparison with the control. However, no significant total colour differences (ΔE) were observed between samples.

Thiobarbituric acid reactive substances (TBARS) was evaluated through malondialdehyde (MDA) (Fig. 4). In all cases, the level of MDA increased over storage. All samples were below the recommended limit over storage, beyond which fish would normally develop an undesirable odour (1–2 μg MDA/g) (Connell, 1990). This fact could be attributed to the presence of carotenoids, which can act as a strong endogenous antioxidant system in rainbow trout (Ojagh, Núñez-Flores, López-Caballero, Montero, & Gómez-Guillén, 2011).

All treatments were effective in comparison with control for

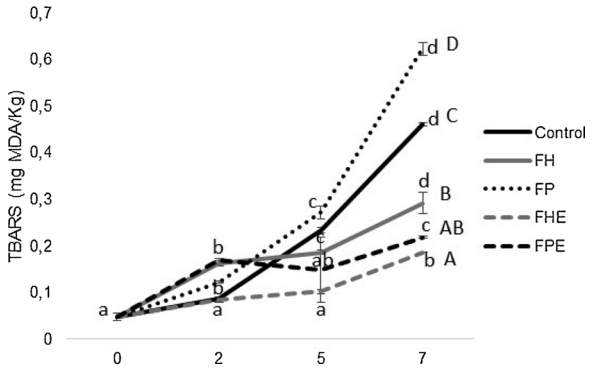


Fig. 4. Thiobarbituric acid reactive substances (TBARS) for fresh fish burgers coated with edible films formulated with *Himanthalia elongate* (FH) and *Palmaria palmate* (FP) seaweed and with extracts of *Himanthalia elongate* (FHE) and *Palmaria palmate* (FPE) and stored at 4 °C for 7 days. Values (mean ± standard deviation, n = 3) followed by different uppercase letter in same column and lowercase letter in same file are significantly different ($p < 0.05$).

reducing the lipid oxidation. Films with seaweed extracts (FHE, FPE) prevented better lipid oxidation than those seaweeds (FH, FP). Nevertheless, *H. elongata* and both seaweed extract films inhibited fish lipid oxidation. Other authors found similar results, Cox and Abu-Ghannan (2013) reported that the addition of *H. elongata* was also effective reducing TBARS in cooked beef burgers, and Yuan et al. (2005) reported *P. palmata* extract inhibited the production of TBARS in linoleic acid emulsion.

Different seaweeds extracts (*Durvillaea antarctica*, *Pyropia columbina, Ulva lactuca*, *Macrocystis piryfera*, *Gracilaria chilensis*), incorporated as covering liquids, have been described by their ability to delay secondary lipid degradation products in canned Atlantic salmon (Ortiz, Vivanco, & Aubourg, 2014). *Fucus vesiculosus* extracts prevented lipid oxidation in fish muscle models (Jónsdóttir et al., 2016; Wang et al., 2010). Brown seaweeds are rich in phlorotannin, which are potent antioxidant *in vitro*. The exact antioxidant mechanism of these phlorotannin components in fish model has not been clarified yet. Phlorotannin compounds must interact with muscle cell membrane, which are the main substrates in lipid oxidation (Jónsdóttir et al., 2016). *Fucus vesiculosus* extracts can inhibit the pro-oxidative effect of haemoglobin due to their high reducing capacity, high DPPH radical scavenging properties and a high oxygen radical absorbance of this seaweed extract (Wang et al., 2010).

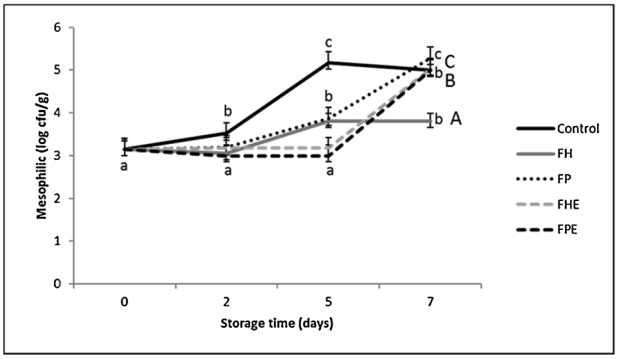
4.3.2. Microbiological analysis

The changes in microbial flora (Total aerobic mesophilic and Total psychotropic bacteria) were shown in Fig. 5. Initially, fish burgers showed total aerobic mesophilic and total psychotropic bacteria counts of 3 and 2 log cfu/g, respectively, which indicated acceptable fish quality. Kakaei and Shahbazi (2016) reported bacterial counts of fresh rainbow trout to be in the range of 3–4 log cfu/g. However, in this study, the mincing process generated a fish product with a larger surface area, which consequently makes it more exposed and susceptible to microbiological, physical and chemical changes during its storage (Roohinejad et al., 2017).

Total aerobic mesophilic (Fig. 5I) increased over storage, with the exception of control samples, which reached the highest counts at day 5. Total aerobic mesophilic counts were maintained constant from this point. However, the acceptable limits for saleability (EC (2005)) might not exceeded for the period of time studied, in total aerobic mesophilic.

The application of edible films (FH, FP, FHE, and FPE) reduced initial total aerobic mesophilic counts, being differences more significant at day 5. Edible films with extracts (FHE, FPE) were more effective than those with seaweeds (FH, FP) in limiting total aerobic mesophilic growth until day 5. Edible film with seaweed extracts (FHE, FPE) could have released seaweed antimicrobial compounds faster than edible film seaweed (FH, FP). However, release kinetics of antimicrobial compounds from seaweeds to food products has been little

I



II

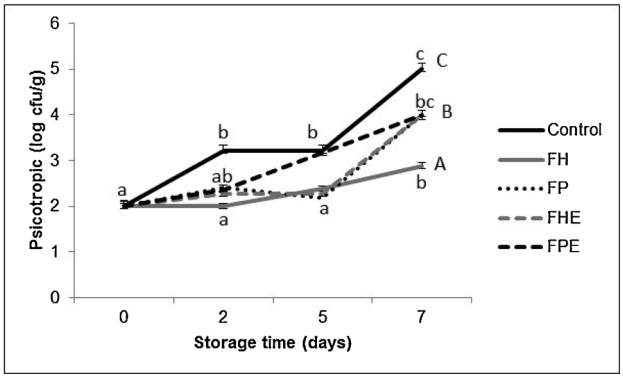


Fig. 5. Total aerobic mesophilic counts (I) and psicotropic (II) for fresh fish burgers coated with edible films formulated with *Himanthalia elongate* (FH) and *Palmaria palmate* (FP) seaweed and with extracts of *Himanthalia elongate* (FHE) and *Palmaria palmate* (FPE) and stored at 4 °C for 7 days. Values (mean ± standard deviation, n = 3) followed by different uppercase letter in same column and lowercase letter in same file are significantly different ($p < 0.05$).

explored. A high migration was found in films of polylactic acid (PLA) with seaweeds, as reported by Rodríguez-Martínez et al. (2016), who observed that the slow migration was associated to a weak retention of the seaweeds in the polymer.

Total aerobic psychrotropic counts (5 °C) were lower than aerobic mesophilic counts (Fig. 5II); similar results were found by Hoel, Jakobsen, and Vadstein (2017). This can be due to the fact that H_2S-producing bacteria, mainly lactic acid bacteria and *Enterobacteriaceae*, as the most important group of fish spoilage microorganisms (Gram & Dalgaard, 2002), have limited growth below 7 °C, and fish spoilage microbial populations can significantly change when different temperature ranges are used (Hoel et al., 2017).

H. elongata seaweed film (FH) was the most effective reducing total aerobic psychrotropic growth over storage. The antimicrobial potential of algae has generally been tested *in vitro*. There is scarce literature on seaweeds as antimicrobial in real food matrices (Pina-Pérez, Rivas, Martínez, & Rodrigo, 2017). Gupta, Rajauria, & Abu-Ghannam, 2010 evaluated *in vitro* the antimicrobial activity of brown Irish edible seaweeds against food pathogenic and food spoilage bacteria. *H. elongata* demonstrated the highest antimicrobial activity in this study. Seaweeds produced secondary metabolites with antimicrobial capacity, such as volatile components (phenols, terpenes), steroids, phlorotannins and lipids (Pina-Pérez et al., 2017). Phlorotannins such as eckol, dieckol, and phloroglucinol in brown seaweeds showed a strong antibacterial activity against number of microorganisms (Eom et al., 2012).

Burgers covered with edible film with *P. palmata* extract (FPE) showed the highest total aerobic psychotropic growth after control burger. This low antimicrobial activity of edible film with *P. palmata* extract could be attributed to a variety of fat-soluble carotenoids, including high levels of lutein, α- and β-carotene and chlorophylls (Dawson, 2007). These carotenoids may have provided significant antimicrobial activity (Pina-Pérez et al., 2017).

In conclusion, edible films formulated with *P. palmata* (FP) and both seaweeds extracts (FPE and FHE) were less effectiveness reducing microbial growth (total aerobic mesophilic, total aerobic psychrotropic) than with *H. elongata* seaweed (FH). There was a limited antimicrobial data for *P. palmata*, but many species of red seaweeds demonstrated moderate *in vitro* antimicrobial activity (Moroney, 2015). A minor antimicrobial activity in edible films with extracts (FHE, FPE) could be attributed to the antimicrobial concentration compounds in the extracts incorporated in the edible films. As previously suggested by Al-Saif, Abdel-Raouf, El-Wazanani, and Aref (2014)) working with seaweed extracts, significantly increased antibacterial activity could be obtained with other solvents than water.

4.3.3. Antioxidant capacity

Edible films were coated in fish fresh burger. The films formulated with seaweed extracts (FHE, FPE) significantly increased the antioxidant capacity of fish burgers when the antioxidant activity was evaluated using ORAC assay (Fig. 6). Antioxidant compounds from extracts seaweeds films (FHE, FPE) were released over storage. Benbettaïeb, Tanner, Cayot, Karbowiak, and Debeaufort (2018)) pointed out that the activity of natural antioxidants (ferulic acid, caffeic acid and tyrosol) from chitosan-fish gelatin edible films can be limited by the release kinetic. The antioxidant compounds from seaweed films were probably released at a higher rate than those consumed due to oxidation.

Both seaweed extracts films (FHE, FPE) reduced the antioxidant loss better than seaweeds films (FH, FP). This higher antioxidant capacity was in accordance with low lipid (TBARS) oxidation markers in these samples (FHE, FPE). During oxidation, antioxidants act in various ways, binding metal ions, scavenging radicals and decomposing peroxides (Sánchez-Alonso, Jiménez-Escrig, Saura-Calixto, & Borderías, 2008).

5. Conclusions

H. enlongata and *P. palmata* were evaluated as active ingredients in chitosan-based edible films. After characterization of the two seaweed extracts, *H. enlongata* showed higher extractable phenolic content and antioxidant capacity, regardless the method used – TEAC, ORAC or FRAP –, than *P. palmata*. Including seaweeds in the formulations

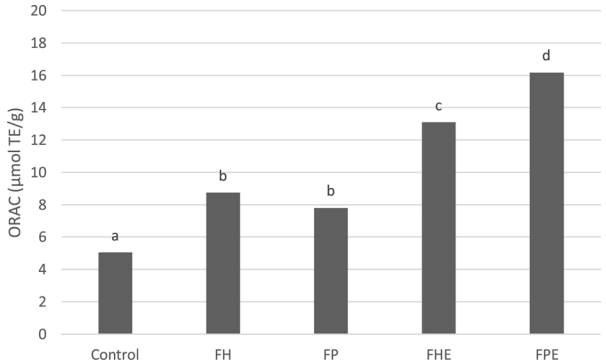


Fig. 6. Effect of edible films on the oxygen radical absorbance capacity (ORAC) for fresh fish burgers coated with edible films formulated with *Himanthalia elongate* (FH) and *Palmaria palmate* (FP) seaweed and with extracts of *Himanthalia elongate* (FHE) and *Palmaria palmate* (FPE) and stored at 4 °C for 7 days. Values (mean ± standard deviation, n = 3) followed by different uppercase letter in same column and lowercase letter in same file are significantly different ($p < 0.05$).

enhanced the thermal properties of the obtained edible films, which can be considered an advantage for their incorporation in packaging. The incorporation of the seaweeds as extracts (FHE, FPE) in the edible films did not improve the antioxidant capacity of these, as compared with the addition of seaweeds directly (FH, FP). Films formulated with *H. elongata* and *P. palmata* edible seaweeds successfully reduced oxidation of fish products, showing also an improvement in microbial spoilage control. Films prepared with *H. enlongata* and *P. palmata* as active ingredients showed potential to be used for fish processed preservation, especially those prepared with *H. elongata.*

Industrial relevance

Plastic Waste is an European strategy to protect the planet, defend our citizens and empower our industries Under this new plans, all plastic packaging on the EU market will be recyclable by 2030, the consumption of single-use plastics will be reduced and the intentional use of micro plastics will be restricted. This strategy will force to create new strategies to extend the shelflife of the fresh product using new barriers to the conventional plastics. In this sense, the incorporation of edible coating with antioxidant properties will provide alternatives to extend fresh products.

Declaration of Competing Interest

There are no conflicts to declare

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