



# Environmentally friendly processing of *Laminaria ochroleuca* for soft food applications with bioactive properties

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## Abstract

Dehydrated *Laminaria ochroleuca* was processed by autohydrolysis with compressed hot water to extract bioactive compounds. Both the whole algae and individual fractions obtained (solid residue and liquor) were characterised to assess its functional properties for future innovative food applications. Purée-like systems were developed by combining ultrasonic and thermal technologies to maximise the antioxidant capacity and were evaluated by determining colour, texture, rheology, syneresis and the presence of bioactive compounds. Overall, the obtained results indicated that *L. ochroleuca* is a valuable resource that can be used as a whole or taking advantage of its bioactive fractions, in a concept of circular economy and sustainability.

**Keywords** Phaeophyta · Edible brown seaweed · Autohydrolysis · Ultrasounds · Waste · Antioxidants

## Introduction

Widely consumed in Asian countries, the edible brown algae *Laminaria ochroleuca* (also known as kombu) is mainly valued in Europe through its extracts, which include alginates, bioactives, and pigments (Fernandes et al. 2016). Alginates are commercially used as thickening, gelling, and stabilising agents in food, cosmetic and pharmaceutical industries. This alga also contains fucoidans that reduce the expression of the pro-inflammatory cytokines and have antioxidant, antimicrobial, and antitumoral properties (Flórez et al. 2017). An alternative could be the development of enriched foods with texture appropriate to special groups of the population, since, by 2050, 1/5 of the world's population will be over 60 years old, numbers that increase to more than 30% in Europe (WHO 2015). Brown algae present a chemically and structurally complex cell wall composed of sulphated and branched

polysaccharides (fucoidan) associated with proteins and various bound ions (e.g. Ca, K) that limits the efficient extraction of the intracellular and wall compounds (Kadam et al. 2015a).

The use of green technologies such as autohydrolysis (AH) and ultrasound-assisted extraction (UAE) could be valuable tools to extract the functional components of this brown alga, towards its integral valorisation. Water-based extraction is food compatible, non-expensive, and environmentally friendly but has low selectivity with low extraction efficiency (Flórez-Fernández et al. 2019). Namely, AH can be performed operating under high temperature and pressure extraction conditions, changes water properties, allowing solubilisation and depolymerisation of compounds present in the matrix of the seaweed (González-López et al. 2012). Since UAE is a useful technique to enhance extraction of bioactive compounds from brown algae (Kadam et al. 2015b), a combination of both techniques mentioned above could be adequate to enhance the yields of polysaccharides with antioxidant and glycosidase inhibitory activities. UAE could provide an economically feasible technique with potential for scale-up and suited for thermolabile compounds (Wan et al. 2015).

Several studies have been performed with *L. ochroleuca* extracts (López-Hortas et al. 2018). However, little is known about the whole algae functional properties, apart from some studies regarding its chemical composition (Sánchez-Machado et al. 2004). In this context, this study aims at the integral valorisation of *L. ochroleuca* using environmentally friendly technologies for the recovery of functional compounds. The chemical and phytochemical properties of the

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obtained extracts as well as the mechanical features of the formulated systems with the whole alga were performed. Potential applications for both extracts and solid residual fractions were outlined.

## Materials and methods

### Materials

Dehydrated *Laminaria ochroleuca* used as raw material was generously provided by Algas Atlánticas Algamar Co. (Pontevedra, Spain). Algae were milled and sieved in order to obtain particle size ranges below 0.25 mm, and between 0.25 and 2.0 mm. For comparison purposes, two commercial references were used, namely, a purée-like food product aimed for the senior population and fresh pasta with spinach filling.

### Subcritical water extraction treatment

The coarse milled fraction (0.25–2 mm) *L. ochroleuca* was subjected to hydrothermal processing (i.e. autohydrolysis) with compressed hot water at 160 °C, using a liquid:solid ratio of 30:1 (w/w), to isolate fucoidan and phlorotannin fractions from the alga (Flórez-Fernández et al. 2019). Four extraction trials were made by placing around 60 g (d.b.) of algae in a pressurised reactor (Parr 4848, USA), operating at about 7.5 atm. Then, a conventional filtration process was used to separate the solid and liquid phases. Both liquor and solid residue were chemically characterised and used to prepare purée-like mixtures (Fig. 1). The yield of each extraction trial was calculated, as well as severity factor (log Ro), i.e. the treatment efficiency, according to Overend and Chornet (1987). The molecular weight cut-off (MWCO) of the liquid phase was performed using an Amicon stirred cell (model 8400, Millipore), using membranes of 100, 50, and 30 kDa. All separated fractions (> 100, 50–100, 30–50, and < 30) were stored at 4 °C until further use.

### Preparation of the purée-like mixtures

The lowest particle size fraction of *L. ochroleuca* (< 0.25 mm) was used to prepare aqueous dispersions based on the results found for other biopolymer-based materials (e.g. Moreira et al. 2014). Several preliminary trials were conducted to find the optimal preparation conditions: alga sample (10–20% w/w, d.b.) was dispersed in water (40–90 °C), under mechanical stirring (Eurostar Digital, IKA-WERKE) at 300 rpm during 10–30 min. The solid residue from AH was thermally processed in the optimal conditions defined for alga samples. For UAE, an ultrasonic bath (P120H, Elma, Germany) operating at 80 Hz for 10–30 min was used and two temperatures (30 and 80 °C) were tested (Fig. 1). In order to not disturb the

formation of the matrix, the samples were prepared directly in individual sealed glass containers (35 mm height, 32 mm diameter), and left at 5 °C for 24 h to ensure full maturation.

## Chemical measurements

### Centesimal composition

Moisture and ash contents (dry basis, d.b.) were determined by gravimetric methods after drying samples at  $105 \pm 2$  °C and incinerating (575 °C, 6 h), respectively. The nitrogen content was assessed by mass spectrometry using a FlashEA 1112 elemental analyser (Thermo Fisher Scientific). A N-protein conversion factor of 5.38 for brown algae (Lourenço et al. 2002) was used to obtain the protein content (d.b.) of the samples.

### Mineral and metals content

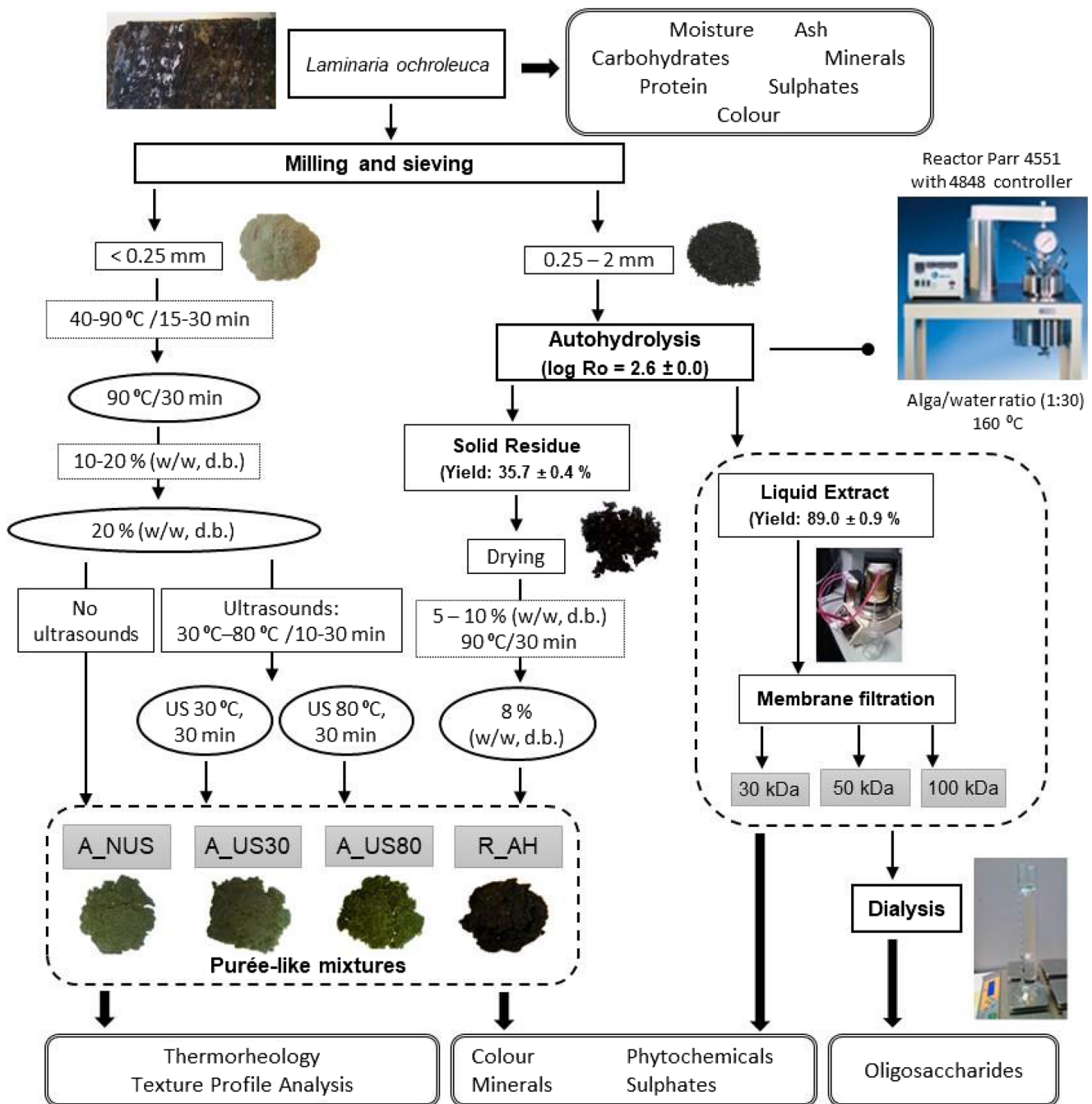
Mineral and metal contents were determined after microwave-assisted (SAVILLEX) acid digestion (80 °C, 6 h). Ca, Mg, K, Na, P, As, Cu, Cd, Pb, Fe, and Zn were analysed by Inductively Coupled Plasma Optical Emission Spectrometry (Optima 4300 DV, Perkin Elmer). Iodine was determined after digestion with tetramethylammonium hydroxide (80 °C, 6 h) by Inductively Coupled Plasma Mass Spectrometry (X Series ICP-MS, Thermo Elemental). Mercury determination was made using Cold Vapor Atomic Absorption Spectrometry according to Fernández-Fernández et al. (2007).

### Sulphate content

Sulphate content was determined at least in triplicate by two different methods. For liquid samples, gelatin-barium chloride procedure was used (Dodgson 1961). Absorbance was measured (500 nm) after incubation at 25 °C, using a standard curve made with K<sub>2</sub>SO<sub>4</sub>. For solid samples, sulphate content was obtained by ionic chromatography method (mobile phase: 3.2 mM sodium carbonate/1 mM sodium bicarbonate at 0.70 mL min<sup>-1</sup>) as previously reported (Gómez-Ordóñez et al. 2010).

### Carbohydrate content

Alga (0.25–2 mm) was hydrolysed with H<sub>2</sub>SO<sub>4</sub> (72 % v/v) in a water bath (30 °C, 60 min). Subsequently, samples were hydrolysed with sulphuric acid (4% v/v) in an autoclave (121 °C, 60 min), and the resulting samples were analysed using High-Performance Liquid Chromatography (HPLC) (Flórez-Fernández et al. 2017).



**Fig. 1** Schematic representation of the present study, showing the preliminary assayed conditions (squares), the optimal conditions adopted (round), and the characterisation performed (double line). The pictures depict *Laminaria ochroleuca* and its milled fractions, the

autohydrolysis reactor and AH products (solid residue and liquor), the dialysis procedure and the end-products (purée-like mixtures). A\_NUS (Alga 20%, 90 °C/30 min), A\_US30 (Alga 20%, 90 °C/30 min, UAE 30 °C/30 min), and A\_US80 (Alga 20%, 90 °C/30 min, UAE 80 °C/30 min)

**Oligosaccharides determination**

The oligosaccharides in the liquid extract were determined by HPLC. First, the salt content in the samples was reduced to 0.2  $\mu\text{S cm}^{-1}$  by dialysis using a membrane tubing (Spectra/Por, Float-A-Lyzer G2, MwCo: 100-500 Da). Then, extracts were filtered through 0.45- $\mu\text{m}$  membranes. Glucose, fucose, rhamnose, formic acid, and acetic acid were analysed using 300  $\times$

7.8 mm Aminex HPX-87H column (BioRad, USA), operating at 60 °C with sulphuric acid (0.003 M, 0.6 mL min<sup>-1</sup>) as mobile phase.

**High-performance size-exclusion chromatography (HPSEC)**

HPSEC was performed to determine the molar mass distribution of the samples, using two 300  $\times$  7.8 mm TSK-Gel

columns in series (G3000PWXL and G2500PWXL, Tosoh Bioscience, Germany) operating at 70 °C, and a 40 × 6 mm PWX-guard column. Milli-Q water with a 0.4 mL min<sup>-1</sup> flow rate was employed as mobile phase. Dextrans below 80,000 g mol<sup>-1</sup> were used as calibration standards.

### Instrumental colour

All measurements were performed using a Minolta CR-400 (Japan) tristimulus colorimeter (five replicates). The colour parameters ( $L^*$ ,  $a^*$ , and  $b^*$ ) were assessed by CIELAB, at 20 ± 1 °C under the same light conditions.  $L^*$  represents the lightness,  $a^*$  the redness or greenness degree, and  $b^*$  the yellowness or blueness degree. The total colour differences ( $\Delta E^*$ ) between samples were determined according to Expression (1):

$$\Delta E^* = \left[ (\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2 \right]^{1/2} \quad (1)$$

### Texture

The texture analysis of the samples was conducted on a texturometer TA-XT2 (Stable MicroSystems, UK). Texture profile analysis was done in a double penetration mode using a P0.25S stainless steel probe (5 kg load cell, 12 mm penetration depth at 1.0 mm s<sup>-1</sup> crosshead speed). From the texturograms (i.e. force versus time plots), the parameters with the greatest ability to discriminate the samples texture were firmness ( $N$ , maximum resistance to the penetration of the probe), cohesiveness (how well the system structure withstands penetration), and adhesiveness ( $N.s$ , resistance of the material when the probe is repressing). All measurements were performed five times at 20 ± 1 °C.

### Thermorheology

Small amplitude oscillatory shear testing was conducted at least in duplicate, and storage ( $G'$ ) and loss ( $G''$ ) moduli were employed to follow the viscoelastic features of purée-like mixtures during the formation process. Suspensions of selected systems were prepared according to the procedure already described and immediately placed into the bottom plate of a controlled stress rheometer (RheoStress 600, Haake, Germany) using cone-plate geometry (diameter 35 mm and angle 2°) and covered with paraffin oil to prevent water loss.

Samples were equilibrated (5 min, 25 °C) before rheological testing. Firstly, stress sweep tests (0.1 to 100 Pa) were run at 1 Hz, and 25 and 90 °C, and the linear viscoelastic region selected: 0.1 to 35 Pa for aqueous seaweed solutions and 0.1 to 65 Pa for purée-like systems. Afterwards, the rheological testing consisted in the following procedure: (1) heating ramp

from 25 to 90 °C (2 °C min<sup>-1</sup>, 1 Hz, 15 Pa) to define the sample melting behaviour; (2) temperature setting (90 °C, 15 min); (3) cooling ramp from 90 to 5 °C (1 °C min<sup>-1</sup>, 1 Hz, 15 Pa) to follow the viscoelastic evolution with temperature; (4) time sweeps (5 °C, 60 min, 1 Hz, 30 Pa) to corroborate the sample maturation kinetics; (5) frequency sweep from 0.1 to 100 Hz (5 °C, 30 Pa) to assess the mechanical spectrum of the sample.

### Bioactive compounds

Five grams of purée-like mixtures were mixed with 95 g of distilled water and incubated at 90 °C for 1 h. After extraction, dispersions were centrifuged (3000×g, 10 min, room temperature) and the supernatant was used as extract. Liquid samples were used as it is. Dry weight of all extracts was determined in order to express the bioactive compounds in the solid samples. The following spectrophotometric methods were performed in an Evolution 201 UV-Vis Spectrophotometer (ThermoScientific), and all analyses were carried out at least in triplicate.

### Scavenging activity

The ABTS radical cation (ABTS<sup>•+</sup>) [2,2-azinobis(3-ethyl-benzothiazoline-6-sulfonate)] scavenging capacity was determined according to Re et al. (1999). Samples (20 µL) and TEAC solution (2 mL) were mixed and incubated (30 °C, 6 min). Phosphate-buffered saline was used as blank. The corresponding absorbance was measured at 734 nm and the results were expressed as equivalents of Trolox (mM).

### Phloroglucinol content

Phloroglucinol content was determined by the Folin–Ciocalteu method as reported by Koivikko et al. (2005). Briefly, sample (500 µL), Folin–Ciocalteu (500 µL, 1 N) and Na<sub>2</sub>CO<sub>3</sub> (1 mL, 20% w/w) were mixed and incubated (45 min in the dark) at 25 °C. Afterwards, the mixture was centrifuged (1600×g, 8 min) before measuring the absorbance at 730 nm.

### Statistical treatment

Statistical analysis was performed using one-way ANOVA (Statistica, version 10.0, StatSoft Inc., USA). Whenever the former analysis exhibited differences amongst means, a Tukey test was conducted to differentiate means with 95% confidence ( $p < 0.05$ ).

## Results and discussion

### Schematic procedure

The schematic procedure adopted in this study is depicted in Fig. 1. Commercial dried *Laminaria ochroleuca* was milled and sieved into two particle size fractions. The one with larger particle size (0.25–2 mm) was used for subcritical water extraction (autohydrolysis) using previously optimised conditions for this alga taking into account the maximum bioactive compounds concentration obtained in the liquid extract (Flórez-Fernández et al. 2019). The smallest particle size fraction (< 0.25 mm) was used to study the effect of different processing treatments on the production of an algae-based structure. Preliminary trials were performed with alga concentrations from 10 to 20% (w/w, d.b.) and temperatures from 40 to 90 °C applied for 15 and 30 min. The best conditions—20%, 90 °C, 30 min—were selected based on the absence of syneresis and in the formation of a visually compact structure that does not flow. According to Kadam et al. (2015b), UAE can be employed to enhance the extraction of bioactive compounds from seaweed. So, preliminary trials were performed applying UAE at 30 and 80 °C for 10 and 30 min. Three purée-like mixtures were selected for further analysis: A\_NUS (Alga 20%, 90 °C/30 min), A\_US30 (Alga 20%, 90 °C/30 min, UAE 30 °C/30 min), and A\_US80 (Alga 20%, 90 °C/30 min, UAE 80 °C/30 min).

On the other hand, from AH processing, two fractions were obtained: a liquid extract (liquor) and a solid residue. Traditionally, only the liquid extract is used, and the solid residue discarded. However, this study intends to valorise the entire algae and its AH fractions, in an economically sustainable logic reinforced by the European Community (EC) directive on circular economy (EC 2015a). So, the solid fraction obtained from AH was characterised and possible applications were studied. The solid residue was dried at 60 °C for 70 h for conservation purposes, and then preliminary trials were performed using 5 to 10% (w/w, d.b.) residue mixed with water and thermally processed in the same way as alga samples. The selected formulation was 8% (w/w, d.b.), 90 °C/30 min (R\_AH). Concerning the liquid AH fraction (liquor), it was purified using membranes with different MWCO: 30 kDa, 50 kDa, and 100 kDa based on the results previously obtained for other brown seaweeds (Álvarez-Viñas et al. 2019).

### Physicochemical and phytochemical characterisation of *L. ochroleuca* and autohydrolysis fractions

The distinct feature of *L. ochroleuca* is its high ash content (Table 1). The mineral content of seaweeds varies according to seaweed species, but other factors also play a relevant role, like oceanic residence time, the geographical place of harvest,

wave exposure, seasonal, annual, environmental and physiological factors, type of processing, and method of mineralisation (Rupérez 2002). Table 1 values are consistent with those reported by Rupérez (2002) and Sánchez-Machado et al. (2004). As compared with other marine species, *Laminaria* spp. have greater ability to extract minerals from the seawater and hence accumulate high amounts of some elements, such as magnesium, calcium, and iodine (Kim and Bhatnagar 2011), presenting here an opportunity for the development of food products with low sodium content as already reported for beef patties with *Undaria pinnatifida* (López-López et al. 2011).

Regarding dried *L. ochroleuca* protein content, the value found is consistent with Sanchez-Machado et al. (2004) results for the same alga species (7.49%, d.b.). Generally, protein content of seaweeds is low when compared with polysaccharides and polyphenols; therefore, they are rarely valued for the functional properties of their proteins (Conde et al. 2013). Amongst seaweeds, Phaeophyta present the lowest protein values (6.4–19.8%, d.b.), although it can vary according to the factors described earlier and the analytical methods used (Cěrná 2011). It is noteworthy that the obtained value for the dried seaweed is lower than the sum of the AH fractions, which can be due to low extraction efficiency in alga sample because of its complex polysaccharide cell wall structure. During subcritical water extraction, proteins are degraded and the water soluble peptides could be extracted (Castro-Puyana et al. 2013) and therefore be present in the AH liquor.

Regarding the solid residue obtained from AH, it still maintains some valuable compounds, namely minerals (K, Ca, and Na). In brown algae, sulphate is a component of fucoidan, a sulphated polysaccharide present in the cell wall of these organisms that protect the algae from desiccation (Rupérez 2002). The complex composition of fucoidans remains uncertain despite the numerous studies about their composition and chemical structure (Martínez-Hernández et al. 2017). It is noteworthy that AH processing promoted the solubilisation of the sulphates since liquor shows a higher content than that of dried *Laminaria*.

Apart from bioaccumulating essential minerals such as Ca, Mg, Fe, and I, seaweeds can also concentrate other elements that can represent a health risk for consumers, namely Pb, Cd, and As. Indeed, due to the adsorption capacity of seaweeds, several studies have been conducted aiming for wastewater treatment and pollution control (Lodeiro et al. 2005). East Asian cultures have traditionally exploited *Laminaria* spp. for food and medicine, and this alga is even listed in the Chinese pharmacopoeia (Kim and Bhatnagar 2011). Unexpectedly, in these countries, which are responsible for most seaweeds' production and consumption as human food, no specific limits have been established regarding its toxic metals content (Circunção et al. 2018). In Western countries,

**Table 1** Chemical composition of *L. ochroleuca* and autohydrolysis products

	<i>L. ochroleuca</i>	Solid residue	Liquor	
	Moisture (g (100 g) <sup>-1</sup> )	9.20 ± 0.07	3.63 ± 0.08	97.81 ± 0.01
	Ash (g (100 g) <sup>-1</sup> , d.b)	35.01 ± 0.31	17.54 ± 0.07	10.28 ± 0.16
	Protein (g (100 g) <sup>-1</sup> , d.b)	9.21 ± 0.12	15.85 ± 0.31	5.61 ± 0.02
	Sulphates (g (100 g) <sup>-1</sup> , d.b)	2.21 ± 0.10	2.11 ± 0.08	14.54 ± 2.2
Minerals (mg kg <sup>-1</sup> )	Calcium (Ca)	7154	14,393	3441
	Potassium (K)	107,314	44,178	175,156
	Magnesium (Mg)	5032	3134	6310
	Sodium (Na)	29,070	11,309	46,956
	Phosphorus (P)	1872	850	2628
	Iodine (I)	4130	1600	6760
	Zinc (Zn)	n.d.	40	n.d.
Heavy metals (mg kg <sup>-1</sup> )	Arsenic (As)	41.3	30.6	43.2
	Cadmium (Cd)	0.7	1.0	0.7
	Copper (Cu)	< 1	2.7	< 1
	Iron (Fe)	22.1	56	3.5
	Lead (Pb)	< 2.2	< 2	< 2.3
	Mercury (Hg)	n.d.	0.065	n.d.

Data are shown as mean ± standard deviation, in dry basis. All data without standard deviations exhibited standard deviations below 2%

there has been an increasing interest in algae research and food development using seaweeds (e.g. López-López et al. 2011; del Olmo et al. 2018). Probably to its gastronomy tradition of using seaweeds for human consumption, on the 1990s, France sets a recommendation on the maximum heavy metal limits: 3 mg kg<sup>-1</sup> As, 0.1 mg kg<sup>-1</sup> Hg, 5 mg kg<sup>-1</sup> Pb (Benoit 2016; AFSSA 2009; Circuncisão et al. 2018). On a regulatory point of view, the Europe legislation only sets maximum allowed levels of Cd (3 mg kg<sup>-1</sup>) and Pb (3 mg kg<sup>-1</sup>) in foodstuffs (Reg. CE 629/2008). This issue is on today's agenda, so in recent years, there has been some efforts made towards a European standardisation on this matter. The European Commission set a recommendation for monitoring the presence of arsenic in food, between 2016 and 2018 to enable an accurate estimation of As exposure (EC 2015b). The FAO/WHO Joint Expert Committee on Food Additives (JECFA) also established for cadmium limits for assumed safe intakes, the provisional tolerable weekly dose (PTWI, µg kg<sup>-1</sup> of body weight in a week) that an adult man can absorb without health damage. These levels have been adjusted throughout the years, and in 2011 were set in 2.5 µg of cadmium kg<sup>-1</sup> body weight in order to ensure a high level of protection of consumers (EFSA 2012).

Our results show that of the analysed heavy metals, only arsenic levels may be of concern in *L. ochroleuca* and its AH fractions, but since a speciation of the chemical forms of arsenic was not performed in this study, only the total arsenic content is presented. Although some studies show that heavy metal levels of brown algae can be reduced by application of AH (e.g. Saravana et al. 2016), we did not observed this

tendency. Moreover, during seaweed metabolic process, inorganic As can be converted into organic forms, less harmful to human health (Taylor et al. 2017).

As already mentioned, membrane technology was used to obtain purified fractions from the AH liquor. Both AH liquor and the membrane fractions were characterised in terms of antioxidant activity (Table 2). The most relevant feature is that the fractioning of AH liquor resulted in a significant ( $p < 0.05$ ) increase in phlorotannin and sulphate contents that showed an increasing trend with decreasing MWCO although without statistical significance. These values are comparable with those obtained by microwave hydrogravity (MHG) from *L. ochroleuca* (López-Hortas et al. 2018). Concerning the scavenging activity, the highest MWCO tested fraction (> 100 kDa) exhibited the largest values. This behaviour nicely matches those reported for other brown seaweeds (*Sargassum muticum*) processed by membranes (Álvarez-Viñas et al. 2019), although the enhancement found here was much lower than that identified in *S. muticum*. A decreasing trend in this parameter was identified to lower membrane size pore, but not with the phenolic content. Even though, the values obtained here were higher than those obtained for the same alga (*L. ochroleuca*) using other green technology as microwave hydrogravity (López-Hortas et al. 2018), reinforcing the formation of novel bioactive compounds using AH.

Regarding the colour parameters of *L. ochroleuca*, AH products and purified fractions (Table S3), there are noticeable differences between *L. ochroleuca* milled fractions, with smaller particle size fraction being lighter, due mainly to the  $L^*$  value, which is directly related to the surface area

**Table 2** Antioxidant activity of AH liquor and purified fractions from *Laminaria ochroleuca*

	Phlorotannins (mg PGE g <sup>-1</sup> extract d.b)	ABTS (mg Trolox g <sup>-1</sup> extract d.b)	Sulphates (mg g <sup>-1</sup> extract d.b)
Liquor	4.65 ± 0.10 <sup>b</sup>	7.05 ± 0.01 <sup>b</sup>	145.4 ± 2.2 <sup>b</sup>
R100	5.72 ± 0.03 <sup>a</sup>	7.91 ± 0.01 <sup>a</sup>	417.9 ± 8.7 <sup>a</sup>
R50	6.05 ± 0.32 <sup>a</sup>	5.42 ± 0.01 <sup>c</sup>	103.5 ± 0.8 <sup>c</sup>
R30	6.18 ± 0.53 <sup>a</sup>	1.81 ± 0.01 <sup>d</sup>	88.4 ± 5.0 <sup>d</sup>
P30	5.62 ± 0.04 <sup>a</sup>	1.55 ± 0.01 <sup>e</sup>	73.3 ± 3.1 <sup>e</sup>

Data are presented as mean ± standard deviation. Different superscript letters in a column show significantly different data values at  $p \leq 0.05$  level

differences. The  $\Delta E^*$  between purified fractions and AH liquor ranges from 3.4 to 6.4, indicating that, apart from R100, the mentioned colour differences are detected by normal human vision (Castellar et al. 2006). The brownish colour of AH liquor is probably due to the formation of new compounds by Maillard and caramelization reactions, which could be favoured by extraction conditions, namely high temperature. These new compounds can possess antioxidant activity as reported by previous studies with plants (e.g. *Rosmarinus officinalis* L., *Thymus vulgaris*), microalgae (*Chlorella vulgaris*), and seaweeds (e.g. *Porphyra* spp., *Undaria pinnatifida*, *Sargassum muticum*, *Saccharina japonica*) (Plaza et al. 2010; Saravana et al. 2016).

### Molecular mass distribution

From the HPSEC spectra of *L. ochroleuca* AH liquor and their corresponding fractions (Figure S1), it is clearly observed that all systems exhibited high molecular weight (> 80 kDa). Liquor, > 100 kDa and 50–100 kDa featured a similar profile. In the latter two fractions, the use of the membranes led to concentrate polymers with higher molecular weight. In the membranes with the lowest pore size, it was observed depolymerization of the polysaccharides presents in the liquor, with peaks close to 80 kDa. These two trends are consistent with phytochemical results.

### Oligosaccharides

The maximum fucose oligosaccharide content (9.84 g (100 g)<sup>-1</sup> extract) was found in the > 100 kDa fraction (Fig. 2) matching with the maximum sulphate content (417.9 mg g<sup>-1</sup> extract) (Table 2). The fucose content was followed by glucose, galactose, mannose, and xylose oligosaccharides in the higher membranes. In contrast, the lower cut-off membranes exhibited a different trend. These results are consistent with the phytochemical results previously explained.

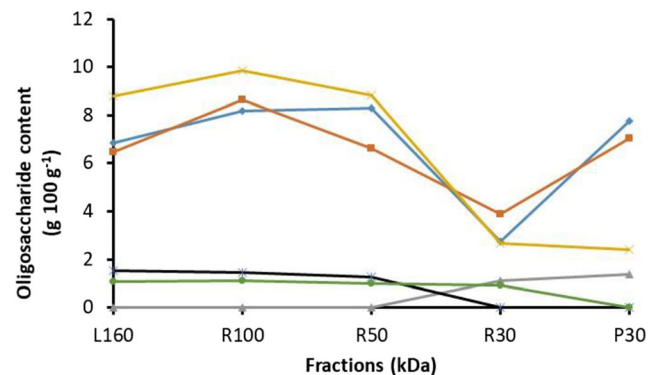
In a recent study, Flórez-Fernandez et al. (2019) performed autohydrolysis at temperatures between 120–220 °C with *L. ochroleuca*. These authors found that the oligosaccharides content continued to increase up to 180 °C, when they start to

decrease, probably due to caramelization and Maillard reactions (Saravana et al. 2016).

## Characterisation of purée-like mixtures

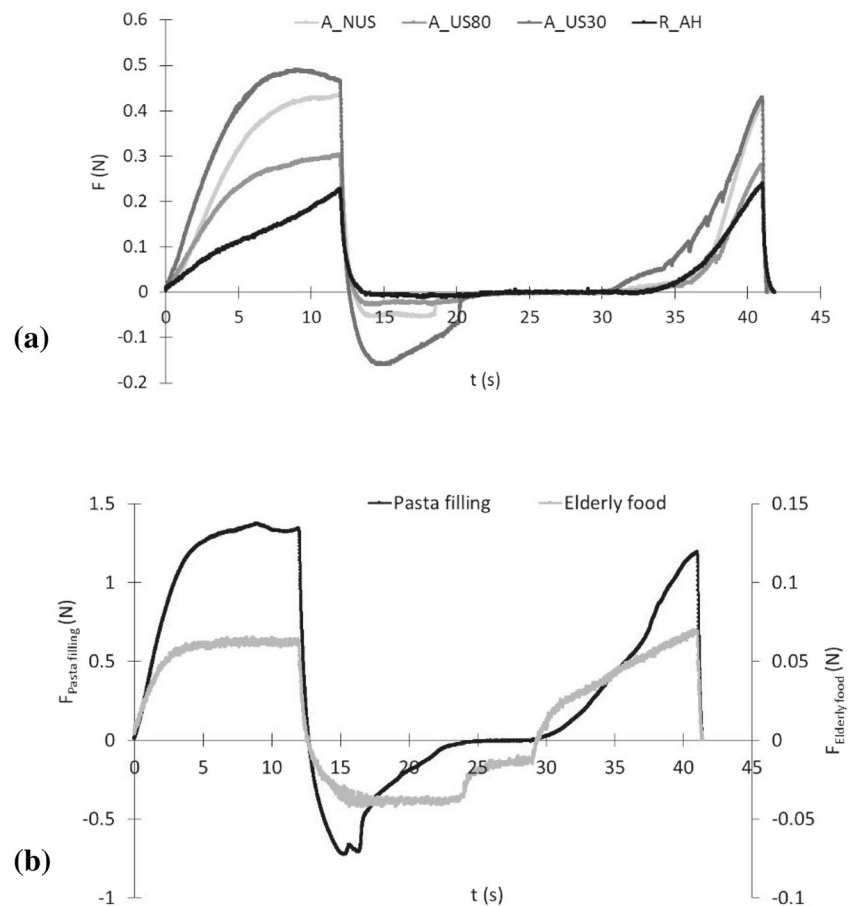
### Texture measurements

The texture profile of the purée-like mixtures and the commercial samples (pasta filling and elderly food) are depicted in Fig. 3 (texture parameters extracted, Table S2). The texture profiles of the alga systems vary according to the UAE treatment (Fig. 3a). As already mentioned, UAE results in the release of bioactive compounds into the medium. This fact translates in a significant adhesiveness gain for the system from -0.31 to -0.92 N.s. This could be associated with a release of phospholipids or glycoproteins from the algae cell wall. However, the same treatment combined with temperature (A\_US80) originated a product significantly less firm and adhesive. This thermal behaviour is consistent with that found for other food matrices, where denaturation and structural changes of proteins were suggested as responsible for the adhesiveness decrease (Pérez-Santaescolástica et al. 2018). These authors also indicated that the combined thermal and ultrasonic cavitation effect could cause loosening of the molecular structure and reduction of molecular nodes.



**Fig. 2** Oligosaccharide and sulphate contents of liquid extract obtained from autohydrolysis (L160) and the corresponding fractions. Symbols: — O-Glucose; — O-Ga+Xy+Mn; — O-Rhamnose; — O-Fucose; — Formic Groups; — Acetyl Groups

**Fig. 3** Texture profile analysis (TPA) for the different tested systems **a** prepared with *L. ochroleuca* brown seaweed (i.e. A\_NUS, A\_US30, A\_US80, R\_AH) as well as **b** those commercially available (i.e. Pasta filling and Elderly food). A\_NUS (Alga 20%, 90 °C/30 min), A\_US30 (Alga 20%, 90 °C/30 min, UAE 30 °C/30 min), and A\_US80 (Alga 20%, 90 °C/30 min, UAE 80 °C/30 min)



Comparing the texture profile of the purée-like systems developed with the commercial food products selected (Fig. 3), A\_US30 is the system that most resembles the pasta filling. Although some differences are observed, especially in their firmness and adhesiveness values (Table S2), it should be pointed out that the systems developed are not finished products, but systems consisting of only alga/residue and water. According to a Finnish Project Report on Food for Seniors (Heiniö et al. 2014), the textures between soft and semi-hard were the most preferred by senior consumers, indicating that some but not too big textural challenges are appreciated. Furthermore, they prefer salty dishes, which can point to the development of the type of products suggested in the present study.

The EC regulation is absent about the use of AH solid residue for food applications. However, the sensorial perception, namely the characteristic aroma, could be an issue. We suggest the use of this raw material in non-food applications, for instance, cosmetic or personal care products.

### Thermorheological measurements

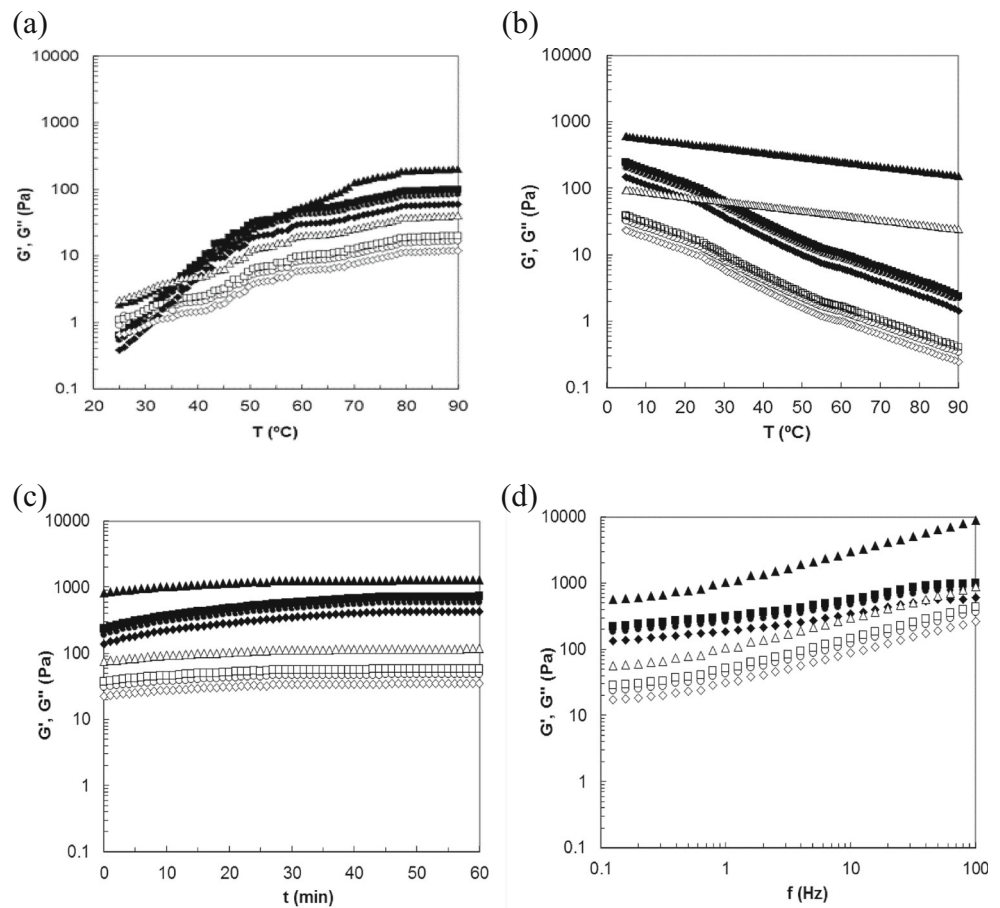
At the beginning of the heating process, all samples showed a predominantly viscous behaviour, with  $G''$

being higher than  $G'$  (Fig. 4a). Although practically no differences related to the sample structure are observed, R\_AH presented a slightly different heating profile than the alga samples. At around 35 °C, there is a crossing of the moduli for all samples that suggests sample structuration due to water uptake from the protein, fibre (*Laminaria japonica*: 50.7 g (100 g)<sup>-1</sup>) or sugars present in the sample (Moreira et al. 2014).

During the cooling process (Fig. 4b), all samples showed a linear increase of both viscoelastic moduli, revealing an increase in structuring as the temperature lowers. The three alga samples showed a similar profile, and different from R\_AH, where the rate of structuring is not so pronounced ( $G'_{90^{\circ}\text{C}} \rightarrow G'_{5^{\circ}\text{C}}$ : 200–600 Pa), whereas for the other samples  $G'$  increases more than 60 times. Fucoidan, the main polysaccharide of *L. ochroleuca*, is very soluble once extracted and the solubility is related to the level of branching, depending on the content of sulphate groups (Cunha and Grenha 2016). During AH processing, algae polysaccharides, namely fucoidan, laminarin, alginate, and its fractions, are released into the liquid extract (Wan et al. 2015), what explains why in R\_AH the degree of structuring is lower than in the alga samples, where these polysaccharides are still present. Although fucoidan does not develop highly viscous solutions



**Fig. 4** Thermorheology of tested systems prepared with *L. ochroleuca* brown seaweed: **a** temperature profiles on heating (25 to 90 °C at 2 °C min<sup>-1</sup>) and **b** cooling (90 to 5 °C at 1 °C min<sup>-1</sup>), **c** time sweep (5 °C), **d** frequency sweep (25 °C). Symbols: circles (A\_NUS), diamonds (A\_US80), squares (A\_US30), triangles (R\_AH), closed (storage modulus, G'), open (loss modulus, G''), A\_NUS (Alga 20%, 90 °C/30 min), A\_US30 (Alga 20%, 90 °C/30 min, UAE 30 °C/30 min), and A\_US80 (Alga 20%, 90 °C/30 min, UAE 80 °C/30 min)



(Rupérez 2002), in fact, it produces aqueous solutions of low apparent viscosity with shear-thinning flow behaviour; alginate forms very viscous solutions (Flórez-Fernández et al. 2019) that can be responsible for the differences obtained between samples.

Regarding the maturation kinetics (Fig. 4c) of the systems, R\_AH is almost instantaneously matured after the cooling process, whereas the alga samples take about 45 min to attain full maturation. At this point, it is clear that A\_US80 shows a slightly weak structure than the other samples, indicating that the temperature in the UAE (and not the UAE) is responsible for the weakening of the structure of this sample. At 80 °C, the proteins in this sample are already fully denatured and not able to form entanglements that result in structural reinforcement.

As depicted in the mechanical spectra (Fig. 4d), these material structure is consistent with pastes, i.e. purée-like systems, what can be explained by the swelling ( $10.20 \pm 0.37$  mL g<sup>-1</sup>, d.b), and water absorption capacity ( $8.93 \pm 0.52$  g g<sup>-1</sup>, d.b) of *Laminaria* (Gómez-Ordóñez et al. 2010). These values are similar to those from *Psyllium* husk, well-known for its water uptake capacity (Raymundo et al. 2014).

Apart from processing conditions in texture and rheology measurements, the results of the mechanical spectra are in agreement with the ones from texture analysis (Fig. 3a).

Although R\_AH presents higher viscoelastic moduli in the mechanical spectrum, it is highly frequency dependent (especially at higher frequency values), indicating a less structured material. This reflects the more cohesive and less firm structure of R\_AH when compared with alga samples (Table S1).

### Colour and syneresis

As expected, in terms of colour (Table S3), R\_AH is distinctively different from the other systems. It presents a darker brownish colour, whereas the algae systems are greener. The  $\Delta E^*$  between the other alga mixtures ranges from 1.26 to 2.45, below the threshold of normal human vision (Castellar et al. 2006). Although none of the samples presented syneresis, R\_AH showed a slight tendency for syneresis with time (not measured). Both of these parameters could be of interest in future applications.

### Bioactive compounds measurements

Purée-like mixtures exhibited a wide range of sulphate content (A\_NUS:  $1.93 \pm 0.6$  g (100 g)<sup>-1</sup>, d.b.) increasing with UAE (A\_US30:  $4.51 \pm 1.3$  g (100 g)<sup>-1</sup>, d.b) and AH (R\_AH:  $13.43 \pm 4.7$  g (100 g)<sup>-1</sup>, d.b.) processing. Note that the sulphate

content of A\_US80 was  $2.46 \pm 0.60 \text{ g (100 g)}^{-1}$ , d.b., lower than all alga pretreated systems. This suggests that some interferences could take place with biopolymers as gelling starch fractions or denatured proteins at  $80 \text{ }^\circ\text{C}$ . In general, the sulphate content of the raw material (Table 1), when compared with the content developed in the purée-like mixtures, exhibited a huge increase. This is probably due to both processing methodologies that favour the solubilisation of compounds and their release into the medium, behaviour consistent with the observed for other brown seaweeds (Flórez-Fernández et al. 2017).

Concerning phlorotannin content, the purée-like mixture pretreatments could allow the formulation of final products with the seaweed or solid residue from AH with values in the same order of magnitude as those obtained in AH liquor ( $4.65 \text{ mg g}^{-1}$  extract), reinforcing the functional potential of the developed matrices. However, the antioxidant activity of these systems could be increased by replacing the water with the AH liquor, since subcritical water extraction allows the formation of new compounds with antioxidant capacity, as already mentioned and reported by several authors (Plaza et al. 2010; González-López et al. 2012). For this purpose, Shibata et al. (2008) suggest that a complex of crude phlorotannins and soybean protein may be useful as a new functional foodstuff with antioxidant and anti-inflammatory activity. To our knowledge, there are no studies with the whole alga processed in the way proposed in this study. These purée-like mixtures could be suited for food applications as a source of minerals or may serve as a food supplement to help meet the recommended daily intakes of some minerals and trace elements (Rupérez 2002). Ageing is also associated with the insufficient intake of micronutrients, which leads to global nutritional deficiency. In this way, the intake of foods rich in vitamins and minerals is recommended for this age group population. Overall, this work represents a preliminary study where proposed purée-like mixtures could be an attractive alternative for specific target population groups. Anyway, before developing any final product formulation, the heavy metal content as arsenic present in the algae formulation should be further studied and surveyed. Even though, previous works (e.g. García-Sartal et al. 2013) indicated that heavy metals present in *L. ochroleuca* exhibited a negligible bioavailability after cooking.

## Conclusions

This approach based on the integral use of *L. ochroleuca* is an alternative to the current production process of chemicals from seaweed focused on a single product (e.g. alginate) and to the more recent cascade biorefinery. Besides, the incorporation of whole algae promotes the simultaneous presence of different components (dietary

fibre, protein, minerals, vitamins, carotenoids, phlorotannins, etc.), with both health-beneficial effects and technological advantages. Future studies are being performed to evaluate the effect of the incorporation of the algae mixtures with gelling agents to expand the spectra of applications.

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