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Drying temperature effect on powder physical properties and aqueous extract characteristics of *Fucus vesiculosus*

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Abstract The effect of air drying temperature on physical properties of dried *Fucus vesiculosus* seaweed and the antiradical capacity and composition of its aqueous extracts were studied. Air drying was performed in a tray dryer employing different temperatures (35, 40, 60 and 75 °C). Dried seaweed (12.2 g water (100 g)$^{-1}$ dry solid) was milled and particle size characterization and colour analysis of obtained powder were performed. Seaweed powder dried at different temperatures showed significant differences regarding colour properties. Lower brightness and yellowness values were determined in samples dried at 50 and 60 °C in comparison to those dried at 35 °C; greenness at 50 °C was enhanced. Nevertheless, particle size distributions of powders were invariant with drying temperature. Dried seaweed powders (<500 μm) were subjected to ultrasound-assisted aqueous extraction (for 4 min and liquid/solid ratio of 30). Total polyphenol content and antioxidant activity (using DPPH• radical scavenging activity) decreased with increasing drying temperature. A linear relationship between both properties was found. Extracts obtained from seaweed dried at 35 °C and sieved to obtain several particle size fractions showed that the maximum polyphenol content was achieved with the intermediate size fractions (80–200 μm). High drying temperatures had a positive effect on alginate extraction yield, but carbohydrate content was not affected (both content referred to raw seaweed powder).

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Introduction

In recent years, there has been an increased interest in natural antioxidants to replace synthetic additives in foods or nutraceuticals. Natural antioxidants not only have the capability to improve oxidative stability, but they can also provide a wide variety of additional health benefits (Wang et al. 2012). Although marine algae are exposed to light and oxygen, causing the formation of free radicals, there is no presence of oxidative damage in the structural components of seaweeds. This suggests that their cells have protective antioxidative defence systems (Jiménez-Escrig et al. 2001). In fact, there are several substances (mainly polysaccharides and polyphenols) present in marine algae that are strongly related to the antioxidant activity (Keyrouz et al. 2011; Hahn et al. 2012).

South Asian countries were the first to introduce seaweeds for their utilization for medicinal and food purposes. Conventionally, the Western world has used marine algae for the production of colloids (agar, carrageenan and alginates). Marine algae are abundant in Europe and have the potential to become an excellent source of bioactive compounds (Kadam et al. 2013). Brown seaweeds represent a suitable supplement and additive for food due to their high nutritional value and the health benefits they can provide. Brown algae have high polyphenol content, with genera like *Ascophyllum* and *Fucus* reaching up to 14 g (100 g)$^{-1}$ dry solid (d. s.) (Holdt & Kraan 2011); particularly, phlorotannins (polyphenols) have been reported to show anti-inflammatory properties and high antioxidant activities (Balboa et al. 2013). Several species such as *Ecklonia cava*, *Ecklonia kurome*, *Fucus vesiculosus*, *Hizika fusiformis* and *Sargassum ringgoldianum* have high

phlorotannin content, which is correlated with the antioxidant activity (Wang et al. 2012).

Fucus vesiculosus is a dominant species of macroalgae in the northern Atlantic Ocean. Hahn et al. (2012) reported the average composition (d. s.) of *Fucus vesiculosus*: 47.8 % carbohydrates (mainly 14.4 % alginate, 12.4 % fucoidan and 12.3 % mannitol), 17.5 % minerals, 10.5 % polyphenols, 10 % proteins, 4.8 % lipids and 9.4 % other components. Other authors determined that this seaweed contains up to 65 % d. s. as polysaccharides (Rioux et al. 2007). Several compounds present in *F. vesiculosus* such as fucoidans (sulfated fucose oligomers), phlorotannins and carotenoids have been reported to show antioxidant activity (Ngo et al. 2011). The current main use of this seaweed in Europe is related to weight loss applications (using raw seaweed or extracts with organic solvents). Besides these uses, further research is needed to increase *F. vesiculosus* applications. This fact makes necessary to study how processing (collection, drying, storage, and extraction, among others) affects these properties.

Drying is one of the most employed industrial operations worldwide as it accounts for about 10–25 % of the total energy consumption in manufacturing processes (Mujumdar 2006). Many bioproducts, as seaweeds, are generally sundried for long periods of time. The current increase of production rates of marine algae requires the application of faster and controlled industrial methods. Air drying conditions are restricted mainly by air temperature and material characteristics. During drying, the solid material can undergo several processes that modify the physical (rehydration, colour loss), chemical (browning reaction, lipid oxidation) and also nutritional (vitamin and protein loss) properties (Bonazzi & Dumoulin 2011). Particularly, colour is the main attribute with respect to the quality of dried materials and can change during drying due to chemical and biochemical reactions. Consequently, colour characteristics, as a measure of the processes promoted during drying, could be related to the properties of the extracts. Some researchers have studied the convective air drying effect on antioxidant activity of different marine algae species (Tello-Ireland et al. 2011; Jiménez-Escrig et al. 2001; Kuda et al. 2005a; Kuda et al. 2005b; Le Lann et al. 2008) but no studies on *F. vesiculosus* were found.

The use of ultrasound technology is widely extended in the food industry. It has been implemented in several large-scale commercial applications such as emulsification, homogenization, extraction, crystallization, etc. It has attracted the attention for its application for the extraction of natural products in a short time. The use of ultrasound improves solvent penetration and disrupts cell walls, releasing its content. Ultrasound-assisted extraction usually increases yields and the quality of the extract and can replace efficiently the traditional technologies to extract bioactive compounds from biomaterials (Picó 2013). Yields and extraction rates increase with smaller particle size, but milling is an energy-intensive operation and must operate without denaturing the material to be extracted and commensurate with separation from the solvent post extraction (Balachandran et al. 2006). Ultrasound-assisted extraction has been used, for example, for the extraction of lycopene from tomatoes (Lianfu & Zelong 2008), anthocyanins from raspberries (Chen et al. 2007), phenolic compounds from citrus peel (Ma et al. 2009) and *Ascophyllum nodosum* and *Laminaria hyperborea* seaweeds (Kadam et al. 2015).

The objectives of the present study are to investigate the effect of air drying temperatures on the physical properties of dried seaweed and its influence on the antioxidant activity and the phytochemical constituents of the aqueous extracts of *F. vesiculosus* seaweed obtained by ultrasound-assisted extraction.

Materials and methods

Raw material and chemicals

Fresh *Fucus vesiculosus* (84.4±2.9 g water (100 g)$^{-1}$ wet solid, w. s.) were collected in the western coast of Galicia, Spain (42.782255 N, −8.929705 W), in October and November of 2014. The seaweed was washed with tap water to remove sand, epiphytes and bugs, and stored (for a maximum of 1 week) at 5 °C until further processing. The analytical grade chemicals used for chemical characterizations were acetone, sodium carbonate, Folin-Ciocalteau reagent, sulphuric acid, phenol, sodium tetraborate, sulfamic acid and 3-hydroxybiphenyl (Panreac, Barcelona, Spain).

Drying

Drying experiments were carried out in a hot air convective tray dryer (Angelantoni, Challenge 250, Italy) at different temperatures (35, 50, 60 and 75 °C) keeping relative humidity (30 %), air velocity (2 m s^{-1}) and initial loading density (14.9±0.1 kg m^{-2}, with a tray area of 0.2 m^2) constant in all experiments. Drying was performed until moisture content of dried seaweed achieved 12.2±0.9 % d. s. The whole seaweed with the exception of the holdfast, which was cut with a thin blade, was employed in the drying experiments. All experiments were carried out at least in duplicate. Samples were briefly withdrawn from the dryer and weighed (Cobos D-6000-CS, ±0.1 g, Spain) every 15 min in the first stages of drying and every hour towards the end. More experimental details were previously reported (Moreira et al. 2015).

Milling

After drying, *F. vesiculosus* was left aerating for 1–2 days to obtain uniform moisture content for dried seaweed. In order to

facilitate milling, dried seaweed was previously ground to a size lower than ~0.5 cm in a Waring laboratory blender (Waring, HGBTWT, USA). Then, it was milled in an ultra-centrifugal mill (Retsch GmbH, ZM200, Germany) using a 500 μm internal sieve. Finally, the milled seaweed with moisture content of 10.5±1.4 % d. s. was stored in polyethylene plastic bags under vacuum with a vacuum packer (Sammic V201, Spain) and stored at 4 °C for further utilization.

Particle size characterization

The analysis of milled seaweed particle size distribution was carried out using sieves (Cisa Cedaceria Industrial, Spain) with different standardized meshes (500, 250, 125, 80, 63 and 40 μm). From particle size distributions, the mass mean diameter D_w, (Eq. (1)), volume mean diameter D_v (Eq. (2)) and surface mean diameter D_s (Eq. (3)) were evaluated for powdered seaweed previously dried at different temperatures.

$$D_w = \sum x_i D_{pi} \tag{1}$$

$$D_v = \sum \left({}^{x_i}/_{D_{pi}} \right)^{-1/3} \tag{2}$$

$$D_s = \frac{1}{\Sigma {}^{\Delta x_i}/_{D_{pi}}} \tag{3}$$

where D_{pi} (μm) is the mean diameter for each fraction and x_i (−) is the weight fraction.

Colorimetric characterization

The surface colour of *F. vesiculosus* seaweed powder and the corresponding particle size fractions were measured using a colorimeter (CR 400, Konica Minolta, Japan) previously calibrated measuring the colour parameters of a standardized white glossy ceramic tile. Colour was evaluated by means of CIELAB coordinates (L*, a* and b*) (CIE 1976). Total colour difference (ΔE*) was calculated taking as reference the milled seaweed previous to sieving (mixture of all fractions), corresponding to the tested temperatures (35, 50, 60 and 75 °C), (Eq. (4)):

$$\Delta E^* = \sqrt{\left(L^* - L_r^*\right)^2 + \left(a^* - a_r^*\right)^2 + \left(b^* - b_r^*\right)^2} \tag{4}$$

where L^* is whiteness ($L^*=0$) or brightness ($L^*=100$), a^* is redness ($a^*>0$) or greenness ($a^*<0$) and b^* is yellowness ($b^*>0$) or blueness ($b^*<0$) and r is the reference value. At least ten colour measures were carried during powder surface scanning.

Ultrasound extraction

Samples of powdered seaweed that were dried at different temperatures (and samples with different particle sizes from seaweed previously dried at 35 °C) were processed with an ultrasound processor (Hielscher, UIP-1000 hdT, Germany) to enhance the extraction of polyphenols and carbohydrates. All experiments were carried out in batch, the procedure starting with a 15-min rehydration step before extraction. Then, extraction operation took place using a 200-mL beaker at controlled temperature (<35 °C) employing a cold water bath to avoid that temperatures increased could affect antioxidant activity. All extractions were performed using water as solvent, excepting in the cases that acetone/water (70:30 *v*/*v*) was used as solvent to obtain reference extracts according to Koivikko et al. (2007). The equipment operated with a frequency of 20 kHz and the irradiation power (<1000 W) was regulated in the ultrasound generator at 80 % amplitude. Preliminary tests (data no shown) were carried out, using *F. vesiculosus* powder formerly dried at 35 °C, varying liquid/solid ratio (20, 30 and 40 *w*/*w*) and contacting time (4, 12, and 20 min) and analyzing polyphenol, carbohydrate and alginate contents, to establish the most adequate extraction conditions for further studies. The selected conditions were 4 min of contact time and 30 g g^{-1} liquid/solid ratio. These conditions provide the highest content in polyphenols, carbohydrates and alginates in the extracts.

Finally, obtained extracts were centrifuged at 12,400 rpm for 15 min using a benchtop centrifuge (SciQuip, Sigma 2 15, UK) and the supernatant obtained was then filtered (0.25 μm) and used for characterization analysis.

Extract characterization

All experimental determinations were carried out at least in triplicate and the corresponding mean values and standard deviations were evaluated.

Total solids content The total solids content in the extracts was determined after sample drying at 104±1 °C. Samples were weighed daily until constant weight was reached after two consecutive measurements (Symons & Morey 1941).

Polyphenol content The quantitative determination of total polyphenol content (TP) was measured as phloroglucinol (PHL) equivalents following a colorimetric method (Singleton & Rossi 1965). This method is based on the absorbance changes of the Folin-Ciocalteu reagent when reacting whit the hydroxyl groups of the polyphenolic substances. TP was evaluated in reference to raw seaweed powder sample (mg PHL (100 g)$^{-1}$ dry sample, TP_w) and also to total solids content in the extract (mg PHL (100 g)$^{-1}$ g dry solids, TP_s).

DPPH scavenging activity The DPPH scavenging activity assay measures the capacity of a system to react with a free radical agent (2, 2-diphenyl-1-picrylhydrazyl, DPPH). It was employed a method previously proposed (Brand-Williams

et al. 1995). In its radical form, DPPH• shows an absorption peak at 515 nm, but upon reduction by an antioxidant (AH) or a radical species (R•), the absorption disappears. As the reaction takes time to fully develop, for the determination of the DPPH scavenging activity, absorbance is measured every 5 min until it reaches the stationary state. Scavenging activity is evaluated by means of (Eq. 5):

$$\text{Scavenging activity}\,(\%) = \frac{(A_0\text{-}A_f)\,100}{A_0} \quad (5)$$

where A_0 (−) is absorbance at time 0 and A_f (−) is the absorbance after one hour.

Carbohydrate content Carbohydrate content of the extracts was determined using the Dubois et al. (1956) method that employs sulfuric acid and phenol as reagents. In the presence of strong acids and heat, carbohydrates form furan derivatives such as furanaldehyde and hydroxymethyl furaldehyde. These compounds react with phenol, leading to the formation of orange-coloured compounds. Furan derivatives from pentoses and hexoses exhibit peaks of light absorbance in the range of 480–490 nm (Brummer & Cui 2005). Samples were evaluated measuring the absorbance read at 485 nm, and glucose was used for the calibration curve. Hence, carbohydrate content was expressed as glucose equivalents (GL) referred to raw seaweed powder sample (mg GL (100 g)$^{-1}$ g dry sample, CHO_w) and also to total solids content in the extract (mg GL (100 g)$^{-1}$ dry solids, CHO_s).

Alginate content Alginate content determination was carried out by means of Blumenkrantz & Asboe-Hansen (1973) method and a further modification by Filisetti-Cozzi & Carpita (1991). The assay consists on the measurement of absorbance at 520 nm of the extracts in the presence of sodium tetraborate in sulphuric acid and *m*-hydroxydiphenil as colour reagent. The Filisetti-Cozzi and Carpita modification introduces the use of sulfamate to reduce the interference of neutral sugars in the measurement (Wrolstad et al. 2005). Glucuronic acid was used as reference for the calibration curve. Therefore, all measures were expressed as glucuronic acid equivalents (GLU) referred to raw seaweed powder sample (mg GLU (100 g)$^{-1}$ dry sample, GLU_w) and also to total solids content in the extract (mg GLU (100 g)$^{-1}$ dry solids, GLU_s).

Statistical analysis

Statistical analyses were performed with IBM SPSS Statistics 20.0.0 software. Levene's test ($P \leq 0.05$) was applied to determine homogeneity of variances of data. For data with non-homogeneity of variances, the Kruskal-Wallis non-parametric test was performed. In the case of homogeneity of variances, when groups of values (k)>2, differences among means were identified by one-factor analysis of variance (ANOVA), followed by the Scheffe's test and considering significant P values≤0.05; when k=2, Student's t test was applied.

Results and discussion

Drying

Fucus vesiculosus seaweed was dried from 84.4±2.9 up to 11.4±0.8 % (w. s.) employing different air drying temperatures (35, 50, 60 and 75 °C). Figure 1 shows the experimental drying kinetics. It can be observed that drying time decreased with increasing drying temperature. In fact, drying at 35 °C exhibited the longest drying time with 25.5±0.5 h, followed by 50 °C with 23.5±0.5 h, and 60 °C and 75 °C required almost the shortest same drying time with 20.0±0.5 h.

Particle size characterization

After drying, algae was milled and sieved in fractions with different particle sizes (from 40 to 500 μm). Table 1 shows the particle size characterization of powders from seaweed dried at different temperatures. In the tested powders assayed, the highest fractions are present at a particle size of 375 μm (38.1–46.7 %). The particle size with the second highest fraction was 162.5 μm (14.1–19.3 %). The minimum yield was obtained for fines or size lower than 40 μm (1.3–4.3 %), and the particle size higher than 500 μm is considered residual, being lower than 1.6 %. Regarding mean diameter values, none of the diameters showed significant differences between temperatures employed during drying. This result seems to indicate that no notorious textural differences are developed

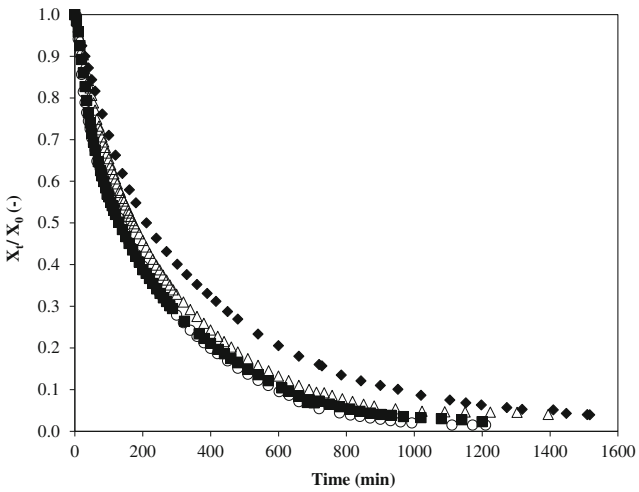

Fig. 1 Experimental drying curves of *F. vesiculosus*. 35 °C (*black diamond*), 50 °C (*triangle*), 60 °C (*black square*) and 75 °C (*circle*); X_t is the moisture content (d. b.) at any time, and X_0 is the initial moisture content of seaweed (d. b.)

Table 1 Particle size distribution and mean diameters for seaweed powder of *F. vesiculosus* previously dried at different temperatures

		Drying temperature (°C)			
		35	50	60	75
Fraction (μm)	D_{pi} (μm)	Mass fraction (%, *w*/*w*)			
>500	500	1.5±0.1	1.1±0.6	1.0±0.3	1.6±0.2
250–500	375	45.8±4.9	40.8±0.5	38.1±5.6	46.7±1.3
200–250	225	9.3±1.2	9.1±2.2	14.3±3.5	9.1±0.4
125–200	162.5	14.1±0.5	16.1±1.9	19.3±4.8	14.6±1.8
80–125	102.5	10.8±0.8	14.3±3.1	10.5±2.0	11.9±2.6
63–80	71.5	7.8±0.6	7.8±2.1	4.2±0.7	6.4±0.9
40–63	51.5	7.5±1.3	9.5±7.7	8.3±0.9	6.6±2.2
<40	30	3.2±0.2	1.3±0.5	4.3±1.1	3.1±1.4
	D_w (μm)	243±20[a]	231±6[a]	234±36[a]	231±10[a]
	D_v (μm)	78±22[a]	91±21[a]	74±5[a]	91±31[a]
	D_s (μm)	133±30[a]	139±19[a]	136±19[a]	146±33[a]

D_{pi} the arithmetic mean diameter of the fraction, D_w the mass mean diameter, Eq. (1), D_v the volume mean diameter, Eq, (2), D_s the surface mean diameter, Eq. (3)

Data are presented as means±standard deviation. Data value of each parameter with different superscript letters in rows are significantly different (Scheffe test; $P \leq 0.05$)

during drying at different conditions. All powders showed similar mean size and surface area; consequently, this last variable should not have any influence on the extraction yield of *F. vesiculosus* compounds.

Colorimetric characterization

The colour of seaweed powders and the corresponding particle size fractions obtained after sieving were measured. Parameter ΔE* of each size fractions was estimated using as reference the colour coordinates of the algae powder prior to sieving. Table 2 shows colour parameter values of powders from seaweeds dried at 35, 50, 60 and 75 °C. Seaweed powders were exhibited in all cases greenness ($a^*<0$) and yellowness ($b^*>0$) predominance. The main change in colour characteristics is found for mixture powders formerly dried at 50 °C due to the attenuation in yellowness (b^*) in comparison with those treated at 35 °C. These results may be linked to the reactions of carotenoids or other pigments, which could result in their degradation, or in the formation of alternative coloured substances or volatile compounds (Landrum 2009). This fact was also noticeable in powders from seaweed dried at 60 °C, which present also low b^* values.

Regarding parameter a^*, powders from seaweed dried at 50 °C revealed a relevant decrease. During dehydration, the tonoplast, the plasmalemma and the chloroplast membrane may suffer structural damage, and as result, a solute loss of chlorophyll and carotenoids, among others components, can occur (Burritt et al. 2002; Oliver et al. 1998). This damage of cell integrity could be related to a loss of antioxidant capacity due to membrane damage which could be enhanced by an increased reactive oxygen species (ROS) production induced by stress conditions (Burritt et al. 2002). Fucoxanthin is an important component of brown algae colouration, and in raw seaweed, it covers the pigmentation of chlorophyll. However, the chlorophyll leaching during drying may expose its colour, and consequently, the parameter a^* drastically decreases. During drying at higher temperatures (>60 °C), the released chlorophyll undergoes degradation reactions. Chlorophylls are easily degraded in the presence of dilute acids, heat, light and oxygen. Along with degradation produced by external agents, chlorophyll is also degraded by chlorophyllase enzyme (Erge et al. 2008). Degradation of the chlorophyll is manifested as yellowing, as it allows the preponderance of carotenoid colouration (Drążkiewicz & Krupa 1991). At room temperature, this enzyme only acts in the presence of high concentrations of organic solvents. However, its optimum activity is found to be within the range of 60–82 °C (Erge et al. 2008). It is difficult to distinguish if chlorophyll breakdown is produced by enzymatic or non-enzymatic reactions but, eventually, they both lead to the formation of non-colourant species (Delgado-Vargas & Paredes-Lopez 2002). Finally, the additional increase of b^* coordinate (yellowness) after drying at 75 °C may be induced by Maillard reactions.

Colour parameter values of size fractions were analysed by means of ANOVA due to homogeneity of variances (Levene's test; $P \geq 0.082$), Table 2. Regarding colour properties of size fractions, parameters L^* and b^* decreased significantly for all systems as mean size increased. Both trends might be related to the presence of still structurally undamaged parts of the alga in the biggest particles. Parameter a^* slightly decreased its absolute value with particle size with exception of powder from seaweed dried at 50 °C, in which very low values were found for fine fractions ($D_p<63$ μm), and above this size, a^* increased with diameter. This behavior has a noticeable influence on the ΔE* parameter for the fractions with the lowest size. Particle size fraction of seaweed powder formerly dried at 60 °C showed brightness, greenness and yellowness loss in all fractions, this effect being likely related with colourant degradation. Finally, the fractions of *F. vesiculosus* powder from seaweed dried at 75 °C displays similar loss in greenness than seaweed dried at 60 °C along with the raise in brightness and yellowness-related parameters (L^*, b^*), which were regarded with browning reactions.

The colour difference (ΔE*) trend showed minimum values at intermediate particle size fractions, with exception of powder formerly dried at 50 °C in which a continuous decrease was observed. The global ΔE* analysis with the particle size fractions for all tested powders indicated that the fractions corresponding to sizes from 63 up to 200 μm showed the lowest average values (ΔE*=7.47±0.31) and for the powders that showed a minimum the lowest average value (ΔE*=5.78±1.78) corresponded to the fractions

Table 2 Colour parameters of powder of *F. vesiculosus* seaweed previously dried at 35, 50, 60 and 75 °C and the corresponding size fractions

		Size fractions (μm)								
T_{drying} (°C)		Powder	<40	40–63	63–80	80–125	125–200	200–250	250–500	>500
35	*L**	56.23±1.94^{c,d,e}	60.56±0.28^{d,e}	61.47±0.61^{e}	58.46±1.02^{d,e}	53.74±1.61^{c,d}	55.36±0.62^{c,d,e}	48.98±0.67^{b,c}	45.22±0.88^{a,b}	39.18±0.95^{a}
	*a**	−2.95±0.17^{a}	−3.04±0.03^{a}	−3.18±0.10^{a}	−3.18±0.09^{a}	−2.85±0.13^{a,b}	−2.90±0.10^{a}	−2.41±0.01^{a,b}	−1.92±0.27^{b,c}	−1.35±0.09^{c}
	*b**	39.19±0.87^{c,d,e}	44.94±0.29^{e}	44.85±0.64^{e}	41.30±0.31^{d,e}	37.98±1.15^{c,d}	37.82±0.35^{c,d}	35.01±0.18^{b,c}	30.87±1.51^{b}	24.12±0.99^{a}
	ΔE*	0	6.53±1.14	7.15±1.16	2.80±1.56	4.29±2.79	2.11±1.37	9.12±0.84	15.30±1.64	23.54±2.59
		Powder	<40	40–63	63–80	80–125	125–200	200–250	250–500	>500
50	*L**	50.68±0.31^{c}	70.95±1.59^{e}	71.36±1.07^{e}	59.34±0.25^{d}	53.75±1.17^{c,d}	47.58±0.50^{b,c}	43.10±0.75^{b}	47.24±1.89^{b,c}	47.59±0.41^{a}
	*a**	−6.40±0.30^{d}	−10.67±0.11^{a}	−11.16±0.06^{a}	−9.77±0.05^{a,b}	−8.63±0.17^{b,c}	−7.11±0.02^{c,d}	−6.11±0.20^{d}	−6.84±0.47^{d}	−6.42±0.48^{d}
	*b**	26.05±0.36^{a,b}	46.93±0.27^{d}	45.96±0.16^{d}	38.80±0.26^{c}	35.55±0.42^{c}	29.61±0.54^{b}	25.79±0.81^{a,b}	28.02±0.70^{a,b}	25.23±1.46^{a,b}
	ΔE*	0	29.41±1.76	29.10±0.53	15.78±0.25	10.23±0.91	4.77±0.64	7.59±1.01	4.00±1.84	3.19±0.29
		Powder	<40	40–63	63–80	80–125	125–200	200–250	250–500	>500
60	*L**	34.45±0.56^{b}	44.83±0.79^{c}	44.30±0.36^{c}	30.03±0.49^{a,b}	26.74±0.64^{a,b}	25.06±0.35^{a}	26.33±3.05^{a,b}	29.60±0.39^{a,b}	24.79±0.87^{a}
	*a**	−2.04±0.04^{a,b}	−2.27±0.04^{a}	−2.41±0.01^{a}	−1.63±0.06^{b,c}	−1.41±0.11^{c}	−1.31±0.03^{c}	−1.38±0.20^{c}	−1.60±0.01^{b,c}	−1.05±0.03^{c}
	*b**	20.39±0.26^{d,e}	29.88±0.41^{f}	27.53±0.27^{f}	21.78±0.28^{e}	18.01±0.34^{c,d}	14.76±0.14^{b,c}	14.15±1.06^{b}	15.04±0.29^{b,c}	10.75±0.39^{a}
	ΔE*	0	14.07±1.43	12.17±0.17	4.69±0.88	8.09±1.20	10.97±0.92	10.30±3.36	7.24±0.28	13.70±0.23
		Powder	<40	40–63	63–80	80–125	125–200	200–250	250–500	>500
75	*L**	56.60±1.34^{e}	64.02±0.38^{f}	55.80±0.80^{e}	50.65±0.78^{d}	48.50±0.65^{d}	46.50±0.26^{c,d}	41.91±0.32^{b,c}	39.16±0.13^{b}	34.11±0.48^{a}
	*a**	−1.86±0.06^{a,b}	−2.04±0.07^{a}	−1.52±0.03^{a,b,c}	−1.27±0.08^{b,c,d}	−1.18±0.08^{c,d}	−1.11±0.04^{c,d}	−0.94±0.09^{c,d,e}	−0.81±0.10^{d,e}	−0.46±0.15^{e}
	*b**	31.40±0.47^{e}	39.60±0.23^{g}	35.23±0.26^{f}	31.54±0.21^{e}	28.89±0.32^{d}	26.45±0.17^{c}	22.66±0.15^{b}	21.68±0.17^{b}	17.95±0.21^{a}
	ΔE*	0	11.22±1.53	3.99±0.39	6.05±0.85	8.73±1.94	11.14±1.92	17.02±1.67	20.01±1.65	26.31±1.58

Data are presented as means±standard deviation. Data value of each parameter with different superscript letters in rows are significantly different (Scheffe test; $P \leq 0.05$)

between 63 and 125 μm. On the other hand, it is noticeable that evaluated volume mean diameters, D_v (74–91 μm), are into the previous interval. This result indicates that this characteristic diameter is the most adequate to estimate approximately the colour properties of the mixtures of seaweed powder formerly dried at 35, 60, and 75 °C. In the case of drying at 50 °C, this relationship was not met, by the reasons previously explained, and the largest size fractions showed the minimum colour differences. Tello-Ireland et al. (2011) also reported that drying *Gracilaria chilensis* at 50 °C resulted in the highest ΔE* value, and drying at higher temperatures showed similar colorimetric coordinates to sample dried at low temperature (35 °C).

Extract characterization

Antioxidant activity

Antioxidant activity of the extracts was evaluated by means of total polyphenol (TP) content and total DPPH• radical scavenging activity. TP values showed homogeneity of variance (Levene's test, P=0.47 for TP_w and P=0.29 for TP_s), and ANOVA analysis was performed. Table 3 shows the influence of drying temperature of algae on the total polyphenol content of the extracts. TP decreased when drying temperature was raised. The significantly highest TP was achieved for the extract made with seaweed dried at 35 °C (1571±76 TP_w, 2940±141 TP_s). TP_{rel} defined as the ratio of TP_w content of the current extract and TP_w from seaweed formerly dried at 35 °C indicated that increasing drying temperature to 50 °C, a reduction of TP_w of 37 % is produced and diminishes up to 54 % when drying temperature of 75 °C is employed. TP_S in the extract showed the same trend. This result implies that differences in TP can be attributed to the effect of drying temperature, and not to a difference in extraction yield.

Table 3 Total polyphenolic (TP) content and total DPPH• radical scavenging activity after 1 h of extracts (4 min contact time and L/S ratio of 30) of *F. vesiculosus* previously dried at different temperatures

T_{drying} (°C)	TP_w	TP_s	TP_{rel}	Scavenging activity (%)
35	1571±76[a]	2940±141[a]	1.00	57.7±3.4[a]
50	983±33[b]	2006±68[b]	0.63	31.0±1.3[b]
60	943±66[b]	2056±143[b]	0.60	30.8±3.2[b]
75	848±73[b]	1670±145[b]	0.54	26.0±1.7[b]

Data are presented as means±standard deviation. Data value of each parameter with different superscript letters in columns are significantly different (Scheffe test; $P \leq 0.05$)

TP_w is referred to sample (mg PHL (100 g)$^{-1}$ dry sample), TP_s is referred to total solids content in the extract (mg PHL (100 g)$^{-1}$ dry solids), TP_{rel} $TP_w(T_i)/TP_w(35\ °C)$

Extractions were also carried out with acetone/water mixture (70/30 *v*/*v*) to achieve the highest extraction yield (Koivikko et al. 2007), replicating the remaining operation conditions. The TP attained was 11,428±1124 TP_w, which is within the interval of 8–13 % of dry matter reported by Ragan & Jensen (1978) and is comparable to TP_w achieved by Díaz-Rubio et al. (2009) in extractions with acetone/water and methanol/water mixtures using *F. vesiculosus*. The maximum TP obtained employing only water as solvent in the ultrasound extraction accounted for 14.4±1 % of TP_w achieved with an acetone/water mixture. Acetone may contribute to a higher degradation of seaweed structure and, therefore, a higher release of these compounds. In addition, polyphenols exhibit a wide difference among their composition and structure and, as a result, in polarity. The use of water as the only solvent allows the extraction of water-soluble polyphenols, while the addition of acetone promotes the extraction of the non-polar fraction as well (López et al. 2011).

On the other hand, the extracts obtained from seaweed dried at 35 °C exhibited the highest radical scavenging activity (57.7±3.4 %), Table 3. As in the case of polyphenols, an increase in drying temperature gave as result a reduction of radical scavenging activity up to the lowest value (26.0±1.7 %) in extracts from the powder of seaweed dried at 75 °C. A linear correlation (R^2>0.998) could be established between TP_s and radical scavenging activity, Eq. (6):

$$\text{Scavenging activity}\,(\%) = 0.044\text{TP}_s - 11.90 \qquad (6)$$

This positive linear relationship between total phenolic content and antioxidant capacity was also found by other authors in fruits (Igual et al. 2010; Bahorun et al. 2004), hulls (Rubilar et al. 2007), leaf extracts (Rubilar et al. 2006) and brown (Connan et al. 2007), green and red seaweeds (Matanjun et al. 2008).

The reduction in polyphenol content and antioxidant activity at high drying temperatures may be due to several factors: release of phenolic compounds bound to the cell wall during drying, thermal degradation by oxidative enzymes, phenolic compounds may rapidly degrade at drying temperatures above 40 °C, binding of polyphenols to other substances (proteins) or alterations in their chemical structure (Gupta et al. 2011; Tello-Ireland et al. 2011; Le Lann et al. 2008). Tello-Ireland et al. (2011) reported the loss of antioxidant activity when drying *Gracilaria chilensis* at high temperatures (70 °C). Gupta et al. (2011) observed a 30 % decrease in TP of *Himanthalia elongata* when dried at 40 °C for 24 h in comparison with fresh seaweed.

Total polyphenol content of aqueous extracts obtained from different particle size fractions of *F. vesiculosus* powder dried at 35 °C are indicated in Table 4. These data showed heterogeneity of variance (Levene's test; P=0.02 for TP_w and P=0.03 for TP_s) so the Kruskal-Wallis test was applied. The size

Table 4 Total polyphenol (TP) content of different particle size fractions of *F. vesiculosus* extracts (contact time of 4 min and L/S ratios of 30) after convective drying at 35 °C

Fraction (μm)	TP_w	TP_s
<80	1222±56[a]	2330±100[a]
80–125	1672±13[b]	3260±20[b]
125–200	1216±75[a]	2410±150[a]
>200	1042±26[c]	2260±60[a]

Data are presented as mean±standard deviation. Significant differences were evaluated by the Kruskal-Wallis test (see comments in the text)

TP_w is referred to sample (mg PHL (100 g)$^{-1}$ dry sample), TP_s is referred to total solids content in the extract mg PHL (100 g)$^{-1}$ dry solids)

fraction between 80 and 125 μm exhibited the significantly highest total polyphenol content (TP_w of 1672±13 mg PHL (100 g)$^{-1}$ dry sample and TP_s of 3260±20 mg PHL (100 g)$^{-1}$ dry solids). The fraction with the significantly lowest TP_w was that with the highest particle diameter (>200 μm). No significant differences were found among the remaining size fractions. The smallest and the biggest fractions exhibited a reduction in total polyphenol content. The first observation may be attributed to the accumulation of inorganic substances, like salts in the smallest fractions. In the largest fractions, seaweed cells could not be totally degraded, and polyphenols, along with other substances, might not have been released indicating that higher extraction times would be necessary for particles this size. Hence, polyphenols are better extracted when intermediate fractions are employed.

Finally, it is noticeable that no relationship was found between the colorimetric properties of dried seaweed powder and antioxidant properties of the extracts. The antioxidant activity decreased continuously with increasing drying temperature, and the colour showed a different trend due to different chemical processes promoted thermally.

Carbohydrate content

The carbohydrate content values (CHO) showed heterogeneity of variances (Levene's test; P=0.002 for CHO_w and P=0.001 for CHO_s) so the Kruskal-Wallis test was applied. For CHO_w, the Kruskal-Wallis test showed no significant differences (P=0.188, mean value of 5204 mg eq. glucose (100 g)$^{-1}$ seaweed) as the effect of drying temperature, Fig. 2. The content of carbohydrates referred to total solids content (CHO_s) was significantly lowest for samples dried at 35 °C (P=0.034) (8217±414 mg eq. glucose (100 g)$^{-1}$). The significantly highest value corresponded to samples dried at 60 °C and 75 °C (12,588±1269 and 12,895±784 mg eq. glucose (100 g)$^{-1}$, respectively). No significant differences in CHO_s values between samples dried at 60 and 75 °C were observed. Although extraction yields referred to raw seaweed powder are similar, there might be compounds already

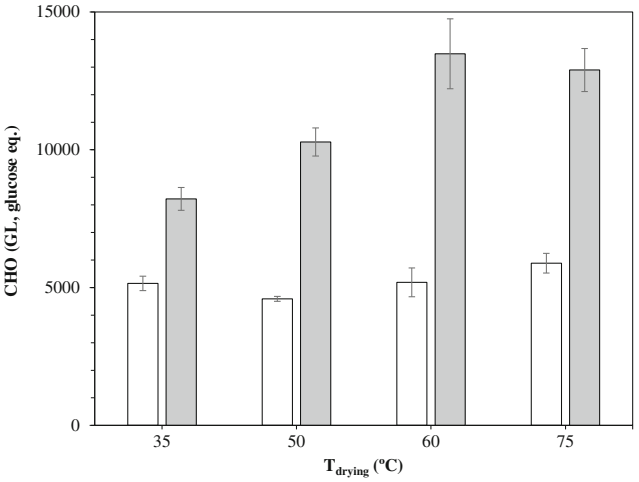

Fig. 2 Effect of drying temperature on carbohydrate content (CHO) of aqueous *F. vesiculosus* extracts (contact time of 4 min and L/S ratio= 30 g g^{-1}): referred to raw powder (mg GL (100 g)$^{-1}$ dry sample, CHO_w (*white square*)), and to total solids content in the extract (mg GL(100 g)$^{-1}$ dry solids, CHO_s (*grey square*))

extracted or degraded during drying at 60 and 75 °C. If these substances are not removed at lower drying temperatures, they are extracted during ultrasound-assisted extraction thus increasing the total solids content of the extract. The extraction with acetone/water (70/30 *v*/*v*) of seaweed dried at 35 °C gave CHO_w of 11,388±156 mg eq. glucose (100 g)$^{-1}$. The aqueous extraction of *F. vesiculosus* seaweed dried at 75 °C exhibited the highest yield in relation to the acetone/water extract (~52 %). As most polysaccharides present in the seaweed are structural substances, a higher drying temperature (75 °C) might have contributed to a better subsequent extraction of carbohydrates.

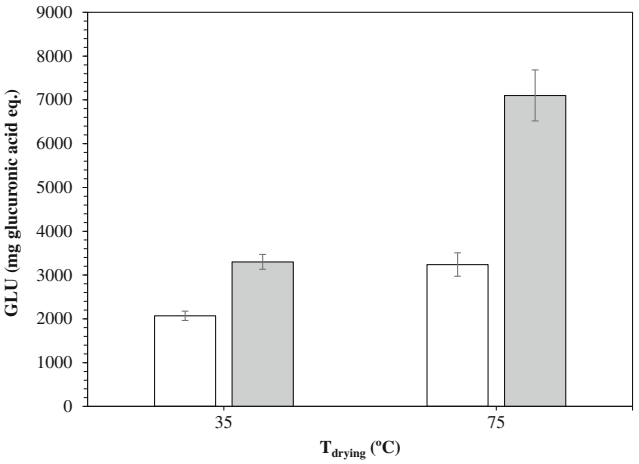

Fig. 3 Effect of drying temperature on alginate content (GLU) of aqueous *F. vesiculosus* extracts (contact time of 4 min and L/S ratios of 30): referred to raw powder, GLU_w (*white square*), and to total solids content, GLU_s (*grey square*)

Alginate content

The alginate content of the extracts were only determined for powders from seaweed dried at 35 and 75 °C, Fig. 3. The value of alginate content of the extracts showed homogeneity of variances (Levene's test; P=0.065 for GLU_w and P=0.056 for GLU_s). In this case due to number of groups, k=2, the Student's test was performed. Alginate content significantly increased with increasing drying temperature from 2070± 107 mg. eq. glucuronic acid (100 g)$^{-1}$ powdered seaweed (GLU_w) to 3240±266 GLU_w (P=0.018) and from 3301± 170 mg eq. glucuronic acid (100 g)$^{-1}$ solid (GLU_s) to 7102 ±583 GLU_s (P=0.006). Tello-Ireland et al. (2011) observed a similar behaviour with red seaweed *G. chilensis* due to, in this case, high temperatures decreased the antioxidant activity of the seaweed but also increased the extraction yield of agar (a structural polysaccharide of red seaweeds). This result seems to indicate that high drying temperature may allow an easier extraction of structural algae polysaccharides. Although alginates can contribute to the scavenging activity, in this case, higher alginate content is associated with lower antioxidant activity. This fact, together with the found relationship between TP and scavenging activity, indicates that the thermal degradation of polyphenols is critical for extract quality.

Conclusions

Particle size distributions of *F. vesiculosus* seaweed after milling were not modified by the drying temperature. Consequently, no significant textural differences were found between seaweeds dried at different temperatures. Seaweed powders exhibited significant differences in colour as the function of drying temperature. Temperatures close to 50 °C causes a greenish colouration of powder. Ground seaweeds previously dried at 35, 60 and 75 °C showed a similar yellow tone. Aqueous ultrasound-assisted extraction is a feasible method to obtain antioxidant compounds from *F. vesiculosus*. Seaweed drying at low temperatures (35 °C) resulted in aqueous extracts with higher phenolic contents and higher DPPH• radical scavenging activities. Higher drying temperatures negatively affected the total phenolic content and the antioxidant activity of extracts. A linear correlation between the phenolic content of extracts dried at different temperatures and their DPPH• radical scavenging activity was found. Nevertheless, no relationship was found between the colour of seaweed powders and antioxidant activity of the corresponding aqueous extracts. Extracts obtained from 80–200 μm particle size fractions of powder obtained from seaweed previously dried at 35 °C showed the highest polyphenol content. Alginate content in the aqueous extracts increased with drying temperature of seaweed, but no significant differences were observed in carbohydrate content, in both cases referred to raw seaweed. As conclusion, *F. vesiculosus* is a suitable raw material to acquire bioactive compounds, which may have applications in the food, cosmetic and pharmaceutical industries. Drying conditions must be controlled to preserve the bioactivity characteristics of the corresponding extracts and to control characteristics as the colour of the meal.

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