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The effect of different drying methods on certain nutritionally important chemical constituents in edible brown seaweeds

Uthman O. Badmus¹ · Mark A. Taggart¹ · Kenneth G. Boyd¹

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Abstract

Seaweeds are potentially a valuable resource for the food, drink and pharmaceutical sectors. The effective utilization of seaweed usually requires post-harvest dehydration in order to prevent decomposition, increase shelf life and aid the extraction of certain chemical constituents. Drying is an expensive, time-consuming and energy-intensive process. Here, the presence of a range of nutritionally important compounds was studied in five brown seaweeds (*Fucus spiralis*, *Laminaria digitata*, *Fucus serratus*, *Halidrys siliquosa*, *Pelvetia canaliculata*) after oven-drying at 40 and 60 °C, freeze-drying and microwave-drying at 385, 540 and 700 W. Antioxidant potential (total flavonoid content, total phenolic content, total antioxidant capacity and radical scavenging activity), soluble protein, lipid, amino acid and fatty acid profiles were determined in each case. Overall, results showed that low-temperature drying, such as freeze-drying and oven-drying at 40 °C, produced products with higher concentrations of nutritionally important chemicals, as well as stronger antioxidant activities. Results suggest that concentrations of nutritionally important chemicals and antioxidant activity are influenced by both the drying treatment and seaweed species used. Where rapid drying techniques are found to be beneficial to levels of specific chemicals, microwave-drying could be a useful alternative to oven-drying, as it helps overcome issues associated with prolonged duration drying (contamination and oxidation). No single drying procedure could be identified as consistently superior for all species or all compounds of interest—indicating that the appropriate drying technique should be selected and optimized for each seaweed species whilst also taking into account potential end-use of the final product.

Keywords Marine algae · Drying techniques · Antioxidant potential · Nutritional chemicals · Seaweed processing

Introduction

Improved insight into links between food choice and human health and wellbeing has led to increased research regarding functional foods (Holdt and Kraan 2011). Functional foods are considered capable of improving human wellbeing and may play a role in preventing chronic conditions like diabetes, hypertension, cardiovascular disease and cancer, as well as providing additional nutritional benefits (Dipplock et al. 1999; Menrad 2003). Studies have indicated that some

seaweeds may be considered functional foods, due to the presence of compounds with proven health benefits (Cornish and Garbary 2010; Suleria et al. 2015). Seaweeds are widely used to produce a range of chemical additives, i.e., for pigmentation (astaxanthin) or to impart hydrocolloid properties (carrageenan, agar and alginate), and are consumed whole as a food, particularly in certain Asian countries (Holdt and Kraan 2011). However, they are not yet a substantial part of many diets in other parts of the world. More recently, studies have examined the incorporation of seaweed as a whole or ingredient within conventional food products and beverages (Roohinejad et al. 2017).

However, fresh seaweed commonly has a high moisture content (of up to 85%, depending on species). Thus, the use of seaweed as an ingredient in the food industry often requires it to be dehydrated before use (Gupta et al. 2011). Drying helps prevent decomposition, increases shelf life and aids the extraction of certain chemical constituents (Ito and Hori 1989). Historically, various drying techniques have been used for foods, including sun-drying, freeze-drying, oven-drying,

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vacuum-drying and, more recently, microwave-drying, which offers the advantage of shorter drying times when compared to other techniques (Zhang et al. 2010). Drying acts to inhibit microbiological activity and slows or stops chemical reactions that cause food to deteriorate (i.e., oxidation or reduction), thus prolonging shelf life (Gupta and Abu-Ghannam 2011; Gupta et al. 2011). Whilst there are different drying techniques available, various factors such as cost, energy consumption, effectiveness and impact on food quality all need to be considered when selecting the most appropriate method (Chen et al. 2016; Stramarkou et al. 2017).

Whilst the main purpose of drying is to preserve the seaweed and extend shelf life, dehydration can also result in negative changes in terms of quality, colour, aroma and the nutritional and phytochemical content of the seaweed (Chan et al. 1997). The quality of seaweed can be reduced if beneficial compounds in the seaweed are lost as a result of drying. For example, phenolic compounds (capable of acting as antioxidants) are often depleted when using certain drying methods, which may then result in reduced antioxidant activity (Ling et al. 2015; Cruces et al. 2016). Other valuable biomolecules such as amino acids, proteins, lipids, fatty acids and other bioactive compounds in seaweeds can also be affected by drying procedures (Chan et al. 1997; Wong and Cheung 2001; Le Lann et al. 2008; Gupta et al. 2011; Ling et al. 2015; Cruces et al. 2016; Neoh et al. 2016; Hamid et al. 2018; Sappati and Nayak 2018; Silva et al. 2019). These studies have demonstrated the value of selecting appropriate drying treatments in order to preserve important bioactive compounds within seaweeds that are destined for use as functional foods.

Of the drying techniques that have been described for seaweed, sun-drying and oven-drying (relatively low-cost options) are the most commonly used (Kadam et al. 2015). However, when sun-drying, seaweed is laid out in the open air in direct sunlight where there is ample opportunity for airborne contamination (i.e., with particulates, microbes, etc.) which may then affect the quality and hygiene of the final product. Alternatively, oven-drying often requires relatively high temperatures for prolonged periods, which may negatively influence the content of heat-labile bioactive compounds and nutrients (Kadam et al. 2015). Previous studies have indicated that freeze-drying may avoid the loss of valuable chemical components in seaweed due to drying, when compared to other conventional methods (Chan et al. 1997; Wong and Cheung 2001), since this avoids the use of elevated temperatures. This is exemplified by Hamid et al. (2018) who compared metabolite profiles in three edible seaweeds (*Cladosiphon okamuranus*, *Saccharina japonica* and *Osmundea pinnatifida*) subjected to varying drying treatments and showed that freeze-dried batches showed higher concentrations of certain metabolites when compared to oven-dried batches. Additionally, a study by Moreira et al. (2017) showed that freeze-dried samples of *Ascophyllum nodosum* had higher

antioxidant activity and contained more phenolic compounds than samples subjected to other drying procedures. However, freeze-drying is an expensive technique to deploy at a large scale, and as a result, it is not widely used in the commercial processing of seaweed. Finally, microwave-drying is an alternative option which may offer rapid drying over short time periods—but again, whilst this has been used in other food sectors (Zhang et al. 2010; Hamrouni-Sellami et al. 2013; Zielinska and Michalska 2016), it is yet to be exploited in the seaweed industry.

Whilst the effects of drying (i.e., oven-drying and freeze-drying) on certain nutritionally important components in some seaweed species have been explored to some degree, the use of time-efficient microwave-drying has received little attention. Likewise, its effect on the final composition of the seaweed product—in relation to different seaweed species—remains virtually unexplored. Here, we considered the effects of freeze-drying, oven-drying (at 40 and 60 °C) and microwave-drying (at 375, 540 and 700 W) on chemical constituents in five common species of brown seaweed, with a particular focus on antioxidant activity and the changes/differences in nutritionally important chemical species in the potential final seaweed product.

Materials and methods

Sampling

Seaweeds (*Fucus serratus*, *Fucus spiralis*, *Halidrys siliquosa*, *Laminaria digitata* and *Pelvetia canaliculata*) were collected from the intertidal zone at Thurso, UK (58.5936° N, 3.5221° W) in January 2017 at low tide. A randomised collection pattern was used, without considering the age and size of the algae. Seaweed samples were rinsed with seawater in the field after collection and were then brought to the laboratory in sealed specimen bags. In the laboratory, samples were thoroughly cleaned with Milli-Q water to ensure they were free from epiphytes, foreign biota, sand and other surface contaminants.

Drying experiments

Three different drying methods were used: (a) conventional oven-drying, (b) freeze-drying and (c) microwave-assisted drying. Samples of each seaweed species (fresh) were divided into six equal portions (100-g wet weight each) and then dried using six different procedures, as shown in Table 1.

For freeze-drying, the seaweed was frozen at atmospheric pressure prior to being placed in a chamber that combines a chilled condenser and a vacuum pump to aid sublimation of water (Mujumdar, 2014). The freeze dryer used was an Edwards Modulyo 4K benchtop unit. For oven-drying,

Table 1 Drying conditions used, showing the duration of drying and temperature (in the case of oven-drying and freeze-drying) and power setting (in the case of microwave-drying)

Drying apparatus	Condition for drying	Drying treatment
Freeze dryer	-60 °C for 5 days	FD
Microwave	385 W for 15 min	M385
Microwave	540 W for 11 min	M540
Microwave	700 W for 8 min	M700
Oven	40 °C for 48 h	OV40
Oven	60 °C for 48 h	OV60

samples were placed into a Thermo Heraeus Scientific T6120 oven and two drying temperatures were used (40 and 60 °C; each for 48 h). For microwave-drying, a conventional domestic microwave was used (Argos MM717CNF) and three combinations of time and power were tested (see Table 1). The oven- and freeze-drying conditions used were based on those described previously by Ling et al. (2015) and on optimisation experiments that determined the times needed to obtain dehydrated material. Drying was considered complete when sufficient drying time resulted in constant dry weight (data not shown). After drying, samples were milled using a kitchen blender to a consistent fine powder. Samples were then stored at 4 °C in glass bottles until analysis.

Analysis of antioxidant potential

Seaweed extraction

This method was adapted from that of Mutton (2012). Briefly, 250 mg of milled sample was weighed into a 15-mL polypropylene centrifuge tube and 5 mL of methanol (analytical grade) was added. The mixture was vortexed (Clifton Cyclone vortex; Nickel-Electro) for 20 s (three times) before sonicating (Grant ultrasonic bath) for 30 min at 55 °C. After sonication, tubes were shaken for 4 h on an orbital shaker (Stuart SSL1) at 300 rpm. Extracts were then centrifuged (Thermo Scientific IEC CL30R centrifuge) at 2500 rpm for 10 min at 24 °C, and the resulting supernatant was decanted into a separate vial. Five millilitres of methanol was then added to the solid residue, and the vortexing and centrifugation procedures repeated. The supernatant was then pooled with that of the first extraction before evaporating to dryness under nitrogen (LV nitrogen manifold; Caliper Life Sciences). The extracts were stored at -20 °C prior to analysis. Before analysis, extracts were re-dissolved in 5 mL of methanol. All extractions were conducted in triplicate.

Radical scavenging potential by DPPH assay

This method was adapted from that of Mutton (2012). Briefly, 100 µL of the formerly mentioned methanol extract was

added to an Eppendorf tube followed by 1 mL of 0.1 mM 2,2-diphenyl-1-picrylhydrazyl hydrate (DPPH) in methanol. Samples were then vortexed before standing at ambient temperature in the dark for 30 min. One hundred microlitres of pure methanol was used as a negative control. After standing, the absorbance of each sample was measured on a UV/Vis spectrophotometer (Camspec M501) at a wavelength of 515 nm. Readings were taken in triplicate (analytical replicates).

Total antioxidant capacity (TAC) by phosphomolybdate reduction assay

This method was based on a slight modification of that described by Prieto et al. (1999). Briefly, 150 µL of seaweed methanol extract (and ascorbic acid standards ranging from 0.1 to 2.5 mg mL⁻¹ for calibration) was added to Eppendorf tubes followed by 1.5 mL of a solution containing: 0.6 M sulphuric acid, 28 mM sodium phosphate (dibasic) and 4 mM ammonium molybdate (1:1:1 v/v/v). Tubes were incubated for 90 min at 95 °C and then allowed to cool to room temperature. Absorbance was then measured at 695 nm.

Total phenolic content by Folin–Ciocalteu assay

The method of Hamrouni-Sellami et al. (2013) was adapted. Briefly, 100 µL of seaweed methanol extract (or gallic acid standard for calibration) was added to a test tube followed by 1 mL of 10% Folin–Ciocalteu reagent (Sigma-Aldrich, UK) in Milli-Q water and 0.8 mL of 7.5% sodium carbonate. Tubes were thoroughly mixed by vortexing and allowed to stand for 1 h at room temperature. Calibration was carried out using gallic acid standards with concentrations ranging from 0.1 to 2 mg mL⁻¹. Standards and blanks were prepared using the same method as for the samples. Absorbance was measured at 765 nm.

Total flavonoid content

This method was adapted from Hamrouni-Sellami et al. (2013). Briefly, 100 µL of seaweed methanol extract or standard ((+)-catechin hydrate) was added to an Eppendorf tube followed by 500 µL of Milli-Q water and 37.5 µL of 5% sodium nitrite. After 6 min, 75 µL of 10% aluminium chloride was added to the tube followed by 250 µL of 1 M sodium hydroxide. Samples and standards were then made up to 1.5 mL with Milli-Q water. Calibrations were carried out using (+)-catechin hydrate standards (concentration range, 0.05–2 mg mL⁻¹). Standards and blanks were prepared using the same method as for the samples. Absorbance was measured at 510 nm.

Protein analysis

This method was modified from that of Gressler et al. (2010). Briefly, 50 mg of seaweed was weighed into an Eppendorf tube, and 990 μL of phosphate buffer (at pH 8) was added, followed by 10 μL of 0.5 M ethylene diamine tetra-acetate (EDTA) and 2 μL of 2-mercaptoethanol. The mixture was vortexed for 30 s, sonicated for 1 h at room temperature before shaking on an orbital shaker at 300 rpm for 15 h, and centrifuging at 7000 rpm for 5 min at 4 °C. After centrifugation, the supernatant was collected. Solutions of bovine serum albumin (BSA) (ranging from 50 to 2000 $\mu\text{g mL}^{-1}$) were prepared for use as standards. Twenty microlitres of each seaweed extract and BSA standard were added to Eppendorf tubes followed by 1 mL of Bradford's reagent (Sigma-Aldrich). Mixtures were vortexed for 30 s and incubated at room temperature for 15 min before measuring absorbance at 595 nm.

Amino acids analysis

Seaweed hydrolysis

This method was modified from Dai et al. (2014). Briefly, 200 mg of dry seaweed was weighed into a 15-mL screw top glass vial, and 5 mL of 6 M HCl added. The vial was flushed with nitrogen to remove air before placing in a pre-heated oven at 110 °C for 24 h. The hydrolysate was allowed to cool to room temperature before centrifuging and filtering through a 0.22- μm syringe filter (Millipore). To 50 μL of hydrolysate, 25 μL of 2.5 mM α -aminobutyric acid in 20 mM HCl (internal standard) was added and made up to 100 μL with Milli-Q water.

Pre-column derivatisation of hydrolysate

Pre-column derivatisation was based on a slight modification of Jones et al. (1981) and Dai et al. (2014), using a mixture of *O*-phthalaldehyde solution (OPA) and 2-mercaptoethanol. The mixture was prepared by dissolving 27 mg of *O*-phthalaldehyde in 500 μL of methanol before adding 50 μL of 2-mercaptoethanol and 5 mL of borate buffer (pH 12.6). One hundred microlitres of this mixture was added to 100 μL of diluted hydrolysate (1:1 v/v), or a mixed amino acid standard (10 μM) or Milli-Q water (blank). Mixtures were vortexed and analysed by HPLC. Samples were prepared in triplicate.

Determination of amino acids

Amino acids were determined using high-performance liquid chromatography (Dionex 3000 Ultimate) with fluorescence detection (RF 2000; Dionex). The analytical method was

adapted from Dai et al. (2014). The analytical column used was a Synergi Max-RP with TMS end capping (reverse phase column(C12), 250-mm length, 4.6 mm ID, 4- μm particle size), with a flow rate of 1 mL min⁻¹. The mobile phase consisted of 25 mM sodium acetate buffer (pH 7.2), methanol and tetrahydrofuran (90.5:9.0:0.5, v/v/v) (solvent A) and 100% methanol (solvent B). The column was eluted using the following gradient: solvent A:B ratio at 0 min was 86:14 was held for 5 min before increasing to 75:25 over 7 min and holding for 6 min, followed by increasing to 50:50 over 7 min and holding for 5 min before increasing to 0:100 over 6 min and holding for 4 min before returning to 86:14 over 45 min. For fluorescence, an excitation wavelength of 340 nm was used along with an emission wavelength of 420 nm. Individual amino acids were identified by matching retention times to those of derivatised individual amino acid standards from prepared from solutions 10 μM of each amino acid in 20 mM HCl (additionally, these were compared with a commercially acquired mixed amino acid standard from Thermo Scientific; product no.20088). Quantification of amino acids was carried out following normalization of the peak area with that of an internal standard (α -aminobutyric acid). Proline and cysteine were not detected; aspartame and aspartic acid were detected as a single peak (aspartic acid) as were glutamine and glutamic acid (which were identified as glutamic acid).

Lipid and fatty acids analysis

Lipid extraction

The method used was a modification of that reported by Schmid et al. (2014). Briefly, 200 mg of dried seaweed was added to a 15-mL glass centrifuge tube and 6 mL of methanol:chloroform (2:1 v/v) added. This was vortexed and then sonicated for 30 min at room temperature. The mixture was shaken for 16 h on an orbital shaker at 300 rpm at room temperature in the dark. After this, 2 mL of chloroform and 3.6 mL of Milli-Q water were added before vortexing and centrifuging at 1000 \times g for 3 min at 4 °C. The organic phase was transferred to a separate tube, and the aqueous phase re-extracted with 4 mL of chloroform (repeating the vortexing and centrifuging steps). Extracts were pooled and evaporated to dryness under nitrogen at 40 °C and then freeze-dried for 15 h to ensure complete drying. The lipid content was determined gravimetrically by subtracting the initial weight of the empty tube from the weight after evaporating the extract to dryness. Dried lipids were stored at -20 °C before transmethylation.

Fatty acid transmethylation and quantification

The analysis of fatty acids that comprise lipids requires transmethylation of the lipids to attain methyl esters of the

fatty acids (which are more volatile than the fatty acids). The method used here was a slight modification of that described by Kumari et al. (2010). Briefly, 30 μL of 5 mg mL^{-1} pentadecanoic acid (dissolved in methanol) was added to the tube containing the dry lipid extract. The mixture was vortexed, and 1 mL of 1% NaOH in methanol was added. The mixture was placed in a pre-heated water bath (with constant stirring) for 15 min at 55 °C; after which, 2 mL of 5% methanolic-HCl was added, and heating continued for another 15 min at 55 °C. After heating, 1 mL of Milli-Q water was added followed by 1 mL of hexane. Solutions were vortexed for 10 s three times and allowed to stand for 30 s. The organic hexane layer was collected and the aqueous phase was re-extracted twice with 1 mL of hexane (repeating the vortex procedure). Extracts were pooled, evaporated to dryness under nitrogen and then re-dissolved in 1 mL of hexane. The extract was then analysed for fatty acids using gas chromatography with mass spectrometry (GC-MS; Shimadzu QP2010); the GC-MS method is described in Table A.1 in the supplementary material. Fatty acids were identified by retention times and comparison of their fragmentation patterns with a commercial fatty acid methyl ester mix (37 component FAME mix from Supelco, USA) and the NIST14 library. The fatty acids were quantified by relating the peak area of the internal standard (pentadecanoic acid) to those of the fatty acids (peak normalisation).

Statistical analysis

One-way ANOVA and Tukey's honestly significant difference (Tukey's HSD) tests were carried out to determine if differences existed in measured parameters due to different drying treatments for each seaweed species, using Minitab 18 software. The significance level was set at $P < 0.05$.

Results

Mass loss from drying during the experiments is shown in Table 2. Mass losses were similar for all species and in all treatments, ranging from 76 to 87%. By species, the highest amount of moisture was lost in *L. digitata* (with losses ranging from 85 to 87%) compared with the other species.

Antioxidant potential

Radical scavenging activity (DPPH inhibition)

Radical scavenging activity (RSA) is reported here in terms of percentage inhibition of DPPH, which is the amount of DPPH radicals quenched compared to a control (methanol). Figure 1 shows that with the exception of *L. digitata* and *H. siliquosa*, RSA varied markedly with drying treatments used. The RSA

of *L. digitata* extracts was similarly consistently low (8.90–14.72%) for all drying treatments. For *H. siliquosa*, consistently high RSA was observed (86.03–92.59%) for all drying treatments. For the other three species (*F. spiralis*, *F. serratus* and *P. canaliculata*), RSA was highest for low-temperature oven-drying (OV40) and freeze-drying treatments and was reduced when higher oven temperatures and microwave treatments were used (more so at high microwave power).

Total antioxidant capacity (TAC)

Figure 2 shows the effect of drying treatments on TAC. Again, *H. siliquosa* shows markedly high TAC values compared to the other four species—regardless of the drying treatment used, with the highest value observed for freeze-dried samples and the lowest for OV40 and OV60. Consistently low TAC was observed for microwave-dried *F. spiralis* and all treatments for *L. digitata*. Freeze-dried and OV40 oven-dried samples of *F. spiralis* and *F. serratus* tended to have higher TAC than the other treatments, as was the case for *P. canaliculata*.

Total phenolic content

Figure 3 shows the effects of drying treatments on total phenolic content (TPC) in methanol extracts of samples. Higher TPC levels were observed for *F. spiralis*, *F. serratus* and *P. canaliculata* when oven-dried at 40 °C (OV40) and freeze-dried (compared to high-temperature oven-drying (OV60) and the microwave treatments). The TPC content of *L. digitata* varied only slightly by treatment and was consistently low. *H. siliquosa* again showed higher TPC than the rest of the species, with considerably higher levels for microwave-dried and freeze-dried batches (compared to OV40 and OV60).

Total flavonoid content

Figure 4 shows the effects of the drying treatments on the total flavonoid content (TFC) in the methanol extracts of the seaweeds. *H. siliquosa* had the highest TFC levels of the five species—with levels highest in low- and high-energy microwave-drying (although M385 was not statistically significant, as shown in Table A.2 in the supplementary material). In all species, no consistent TFC patterns (with drying method) could be observed. For *H. siliquosa* and *P. canaliculata*, higher TFC was observed for certain microwave-dried batches, while for the two *Fucus* species, TFC levels were higher in the freeze-dried batch and/or the OV40 batch. In *L. digitata*, low values that varied only slightly between treatments were seen.

Table 2 Mass loss (in %) in *F. serratus*, *F. spiralis*, *H. siliquosa*, *L. digitata* and *P. canaliculata* after freeze-drying (FD), oven-drying at 40 °C (OV40) and 60 °C (OV60) and microwave-drying at 385 W (M385), 540 W (M540) and 700 W (M700)

Drying treatments	Moisture content (%)				
	<i>F. serratus</i>	<i>F. spiralis</i>	<i>H. siliquosa</i>	<i>L. digitata</i>	<i>P. canaliculata</i>
FD	80.00	79.00	78.00	86.32	76.24
M385	77.14	80.95	81.37	85.15	80.20
M540	79.05	80.20	79.61	86.00	80.00
M700	78.22	76.47	78.00	87.38	77.27
OV40	82.18	81.37	79.50	85.00	80.03
OV60	82.35	80.20	78.50	86.61	80.46

Soluble protein and amino acid content

Soluble protein

The effects of drying on soluble protein are displayed in Fig. 5. All seaweed species showed the highest protein content in freeze-dried batches with the exception of *L. digitata*, wherein levels were consistently low (< 1%). The protein content of *F. spiralis* and *P. canaliculata* was also higher after low-temperature oven-drying (OV40) when compared to high-temperature oven-drying (OV60) and microwave treatments.

Amino acid content

For each sample, amino acids were identified and quantified (Table A.3 in the supplementary material) before determining total amino acid (TAA), essential amino acid (EAA) and non-essential amino acid (NEAA) content. The EAA content is the sum of threonine, valine, methionine, lysine, isoleucine, leucine and phenylalanine and the NEAA is the sum of aspartame, glutamine, serine, glycine, histidine, arginine, alanine and tyrosine. Additionally, the ratio of the NEAA to EAA was determined. The results are shown in Fig. 6. For four of the five seaweed species, *H. siliquosa* being the exception, the highest level of

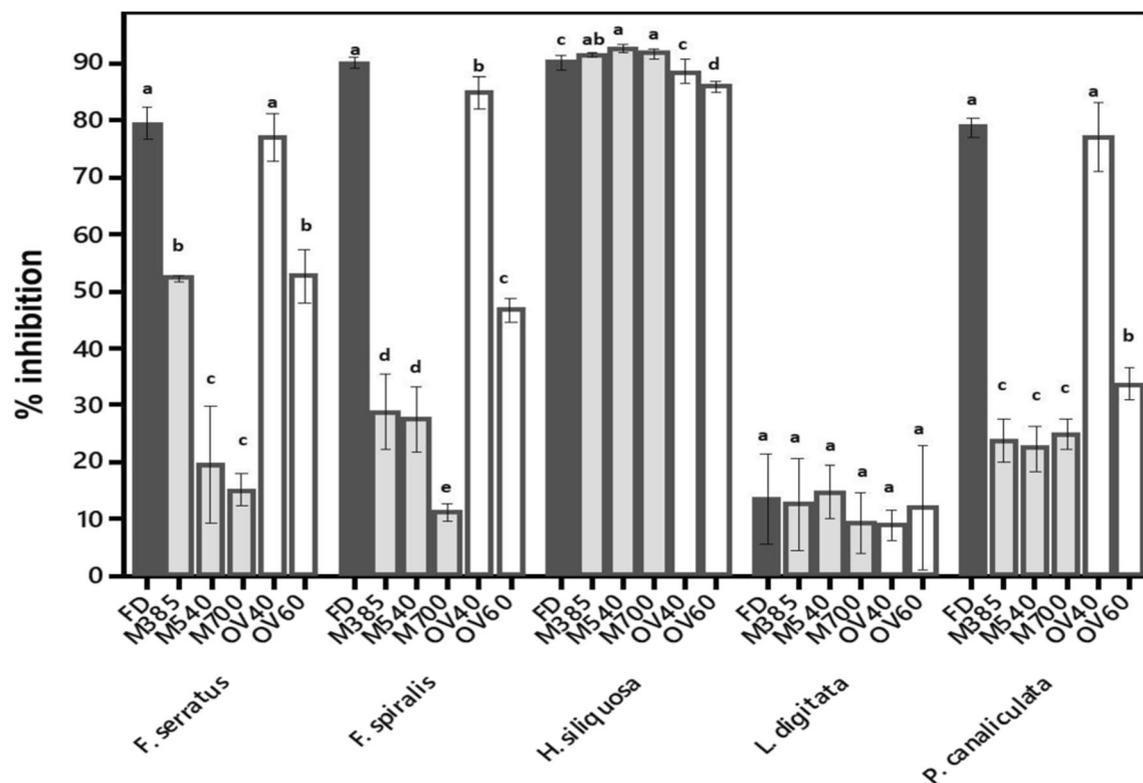


Fig. 1 Radical scavenging activity (% inhibition) of methanol extracts of *F. serratus*, *F. spiralis*, *H. siliquosa*, *L. digitata* and *P. canaliculata* subjected to freeze-drying (FD—black shading), oven-drying (no shading) at 40 °C (OV40) and 60 °C (OV60) and microwave-drying (grey

shading) at 385 W (M385), 540 W (M540) and 700 W (M700). All data points are means of triplicate analyses (independent analytical replicates) and error bars indicate standard deviation. Bars with different letters are significantly different ($P \leq 0.05$; Tukey)

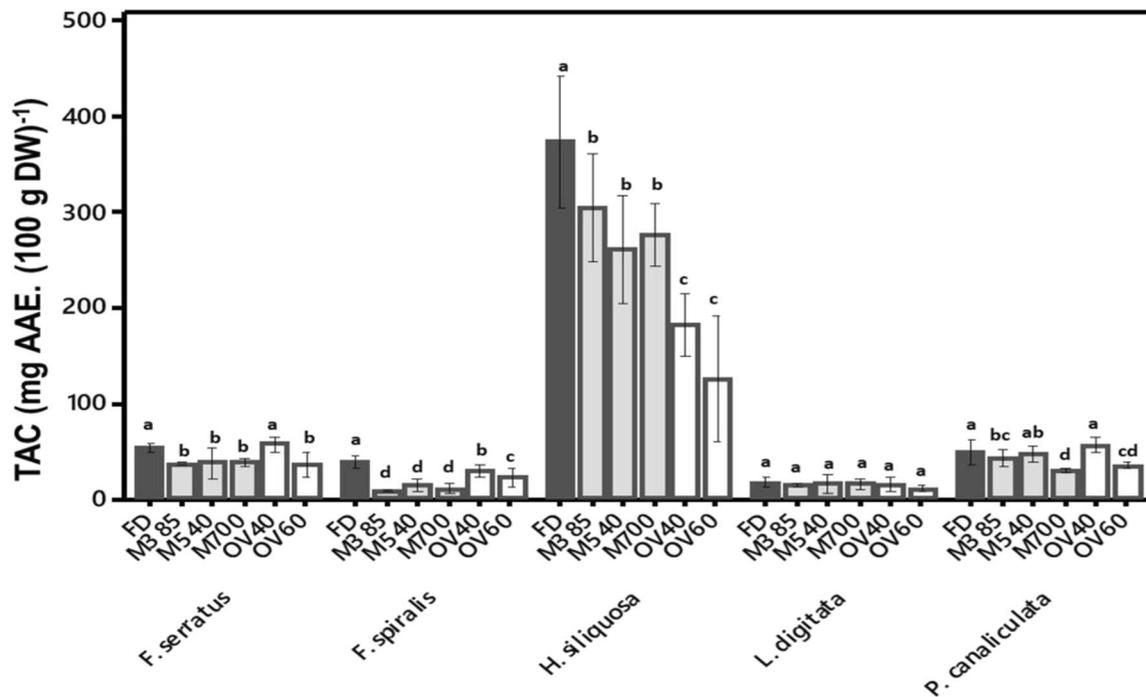


Fig. 2 Total antioxidant capacity (mg AAE (100 g DW)⁻¹) in methanol extracts of *F. serratus*, *F. spiralis*, *H. siliquosa*, *L. digitata* and *P. canaliculata* subjected to freeze-drying (FD—black shading), oven-drying (no shading) at 40 °C (OV40) and 60 °C (OV60) and microwave-drying (grey shading) at 385 W (M385), 540 W (M540) and 700 W (M700). All data points are means of triplicate analyses (independent analytical replicates) and error bars indicate standard deviation. Bars with different letters are significantly different ($P \leq 0.05$; Tukey)

and microwave-drying (grey bars) at 385 W (M385), 540 W (M540) and 700 W (M700). All data points are means of triplicate analyses (independent analytical replicates) and error bars indicate standard deviation. Bars with different letters are significantly different ($P \leq 0.05$; Tukey)

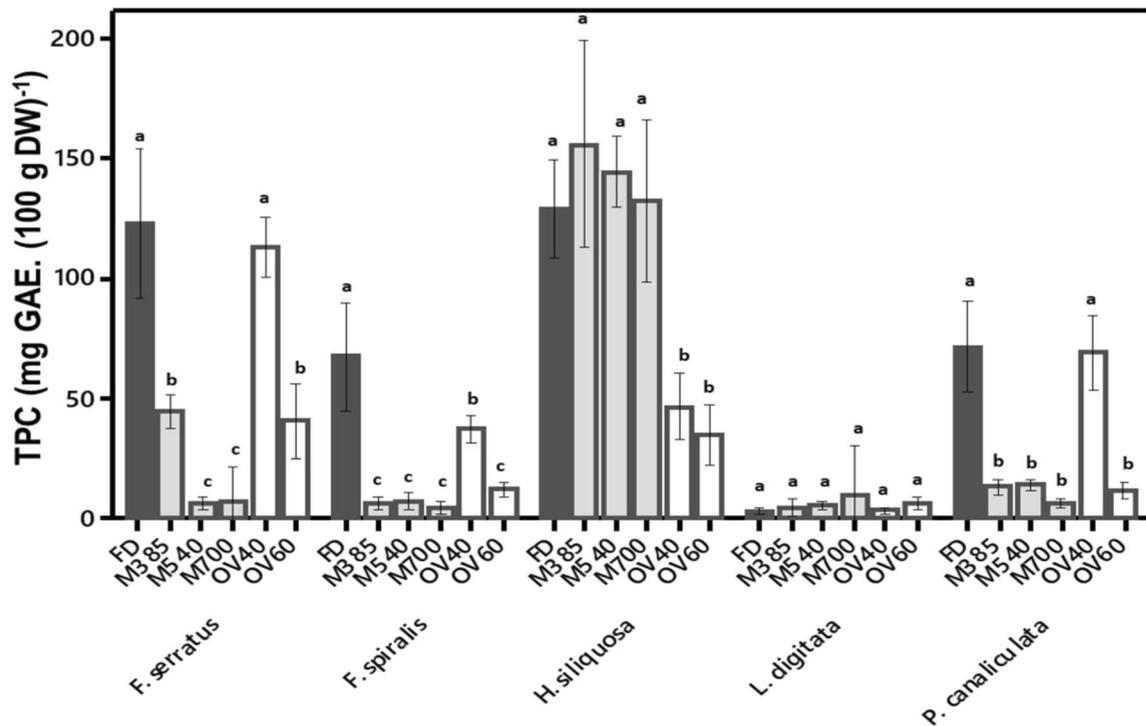


Fig. 3 Total phenolic contents (mg GAE (100 g DW)⁻¹) in methanol extracts of *F. serratus*, *F. spiralis*, *H. siliquosa*, *L. digitata* and *P. canaliculata* subjected to freeze-drying (FD—black shading), oven-drying (no shading) at 40 °C (OV40) and 60 °C (OV60) and microwave-drying (grey shading) at 385 W (M385), 540 W (M540)

and 700 W (M700). All data points are means of triplicate analyses (independent analytical replicates) and error bars indicate standard deviation. Bars with different letters are significantly different ($P \leq 0.05$; Tukey)

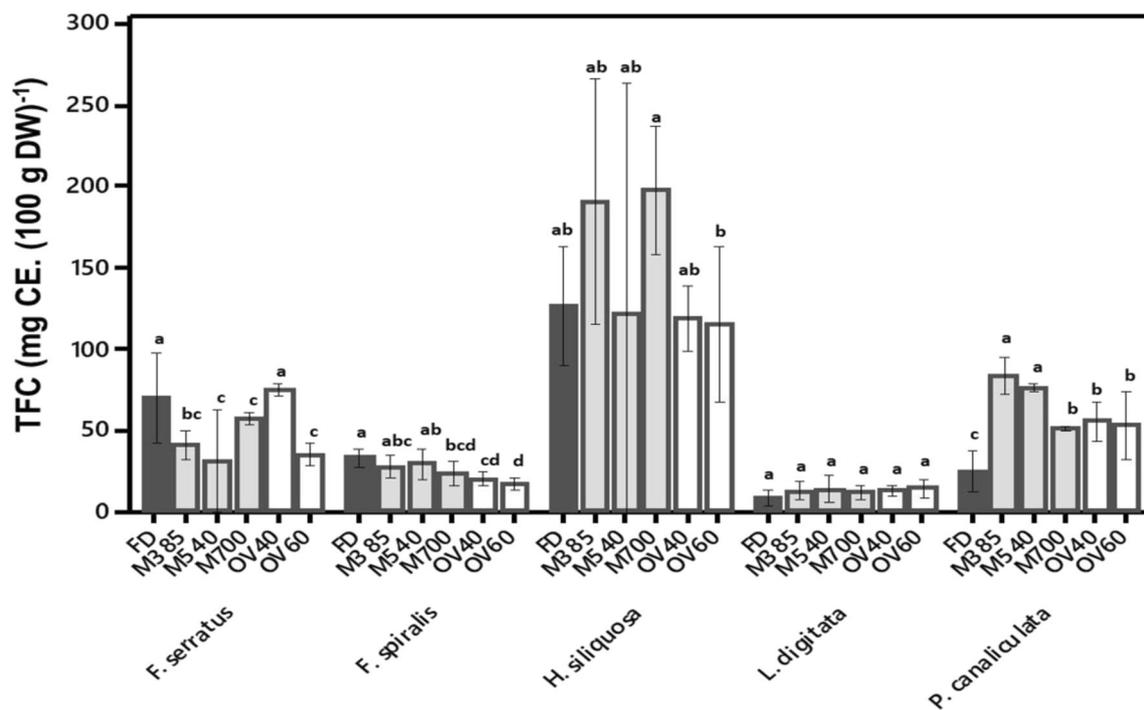


Fig. 4 Total flavonoid contents ($\text{mg CE (100 g DW)}^{-1}$) in methanol extracts of *F. serratus*, *F. spiralis*, *H. siliquosa*, *L. digitata* and *P. canaliculata* subjected to freeze-drying (FD—black shading), oven-drying (no shading) at 40 °C (OV40) and 60 °C (OV60) and microwave-drying (grey shading) at 385 W (M385), 540 W (M540)

and 700 W (M700). All data points are means of triplicate analyses (independent analytical replicates) and error bars indicate standard deviation. Bars with different letters are significantly different ($P \leq 0.05$; Tukey)

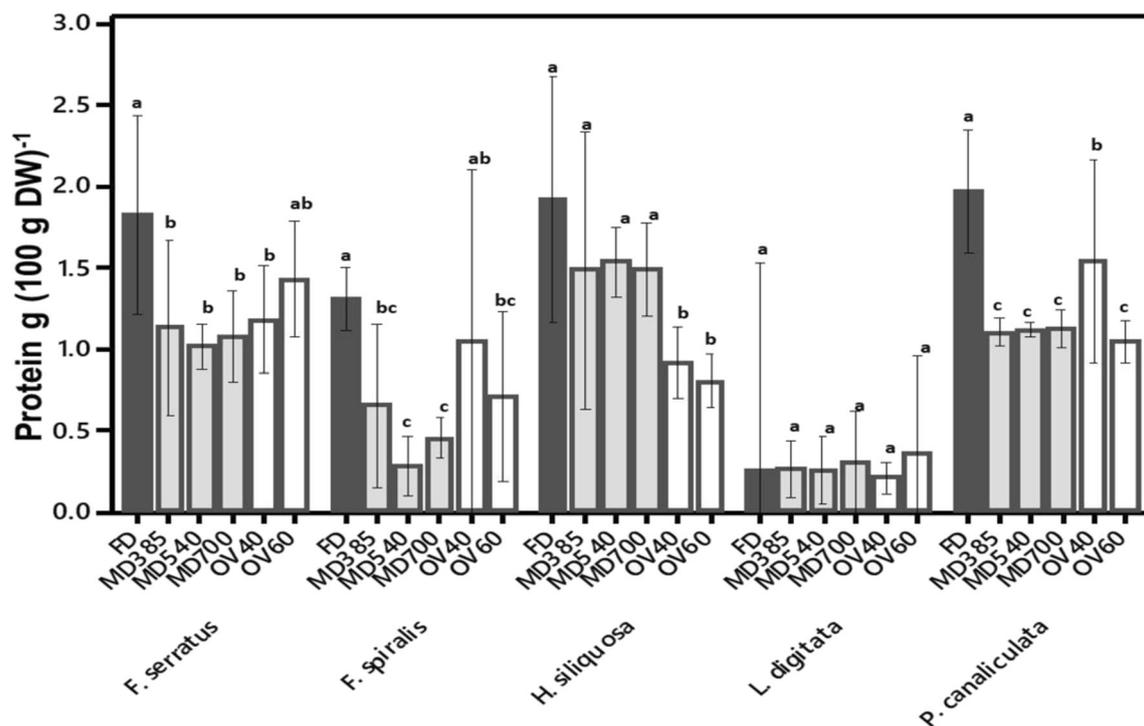


Fig. 5 Soluble protein contents (in g (100 g DW)^{-1}) in *F. serratus*, *F. spiralis*, *H. siliquosa*, *L. digitata* and *P. canaliculata* subjected to freeze-drying (FD—black shading), oven-drying (no shading) at 40 °C (OV40) and 60 °C (OV60) and microwave-drying (grey shading) at

385 W (M385), 540 W (M540) and 700 W (M700). All data points are means of triplicate analyses (independent analytical replicates) and error bars indicate standard deviation. Bars with different letters are significantly different ($P \leq 0.05$; Tukey)

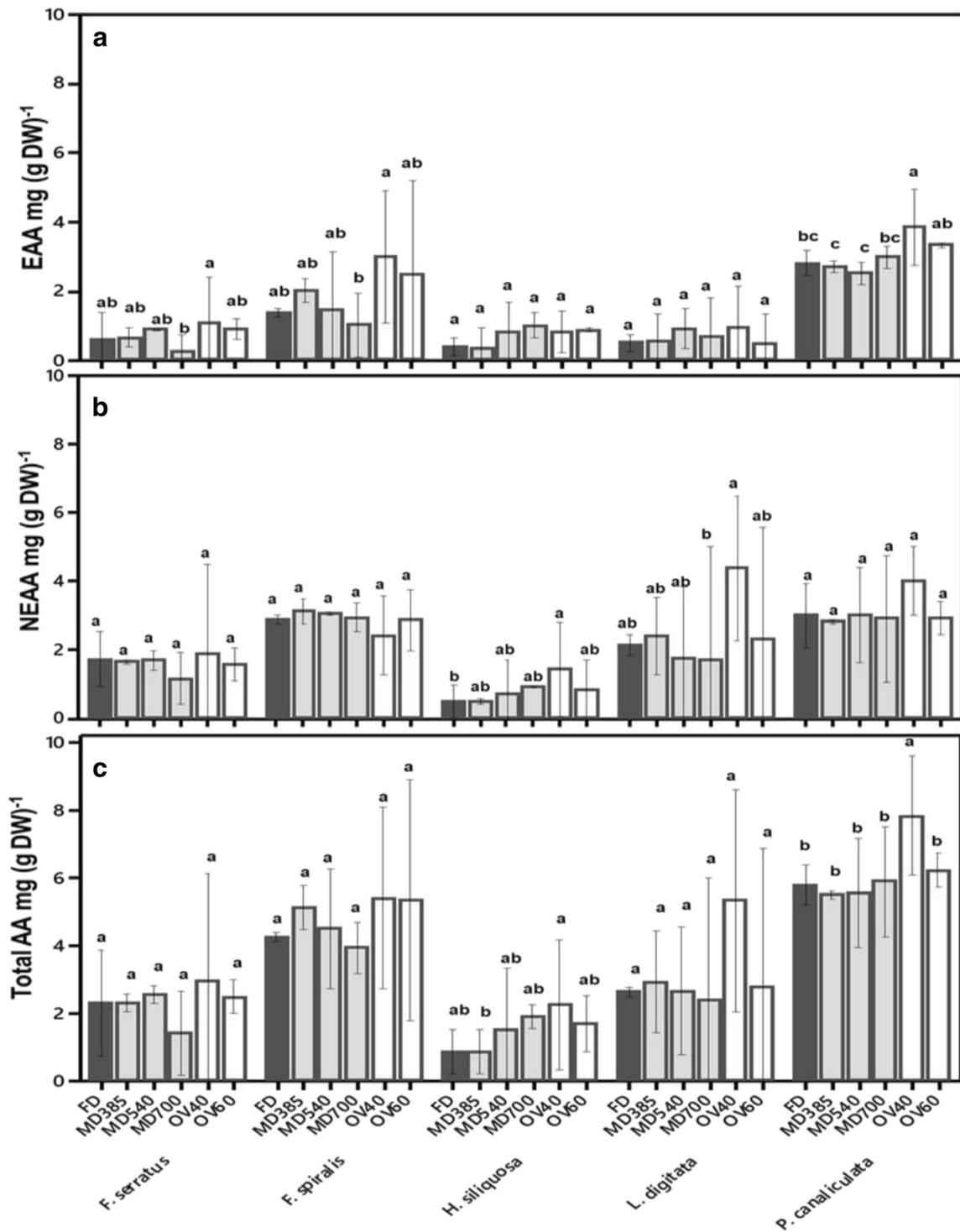


Fig. 6 Amino acid contents in *F. serratus*, *F. spiralis*, *H. siliquosa*, *L. digitata* and *P. canaliculata* subjected to freeze-drying (FD – black shading), oven-drying (no shading) at 40 °C (OV40) and 60 °C (OV60) and microwave-drying (grey shading) at 385 W (M385), 540 W (M540) and 700 W (M700). (a) Total essential amino acid (EAA) (in mg (g DW)⁻¹); (b) total non-essential amino acid (NEAA) (in mg (g DW)⁻¹);

(c) total amino acid (TAA) (in mg (g DW)⁻¹). All data points are means of triplicate analyses (independent analytical replicates) and error bars indicate standard deviation. Bars with different letters are significantly different ($P \leq 0.05$; Tukey). Statistical data are displayed in Table A.3 in the supplementary material

TAA and EAA resulted from low-temperature oven-drying (OV40). A similar pattern is observed for the NEAA, except in the case of *F. spiralis* that showed higher values for the

microwave-dried batches. EAA/NEAA ratio is used to determine the quality of the amino acid content (Stévant et al. 2018) with a higher ratio suggesting a relatively high EAA content, and

therefore better protein quality. Consistent trends in this data are not evident, for example for the two *Fucus* species the highest ratios was found in the OV40 treated samples, while M540 and M700 provided high ratios for *H. siliquosa* and *L. digitata* (alongside OV60 for *H. siliquosa*). For *P. canaliculata*, there was little change in the ratio across the different drying treatments.

Lipid and fatty acid content

Lipid concentration

Lipid content data in relation to drying treatments is displayed in Fig. 7. All species tended to show the highest lipid content in microwave-dried batches, except for *L. digitata* where OV40 gave marginally higher results. In *H. siliquosa* and *P. canaliculata*, the lowest lipid content was observed in freeze-dried batches. In contrast, the lowest lipid levels in the two *Fucus* species were found in oven-dried batches.

Fatty acid profiles

For each sample, fatty acids were identified and quantified (Table A.4 in the supplementary material) before determining the quantities of saturated fatty acids

(SFAs), monounsaturated fatty acids (MUFAs), polyunsaturated fatty acids (PUFAs) and the index ratio of n6 to n3. The effect of drying treatments on fatty acids is displayed in Fig. 8.

The C16, C18:1 and C20:4n6 fatty acids were the predominant fatty acids in the SFA, MUFA and PUFA classes respectively for all seaweeds (see Table A4). In *L. digitata* and *H. siliquosa*, notably higher SFA levels occurred in FD and OV40 samples. Limited SFA differences were seen in the other three species. In terms of MUFA, *F. spiralis* and *L. digitata* showed the highest MUFA content following low-temperature oven-drying (OV40) and/or FD or M385 treatment. For the PUFA levels, *L. digitata* and *H. siliquosa* show higher PUFA contents after microwave treatments, while *F. spiralis* and *F. serratus* showed the highest PUFA after FD. The PUFA levels in *P. canaliculata* showed no significant difference between drying treatments. The ratio of the total omega-6 fatty acid to total omega-3 (n6/n3) is often used to determine the quality of the fatty acid profile, with values in the range of 1 to 2 considered ideal for the prevention of obesity (Simopoulos 2016). For three of the five species studied, the highest ratio for n6/n3 occurred as a result of low-temperature oven-drying (OV40).

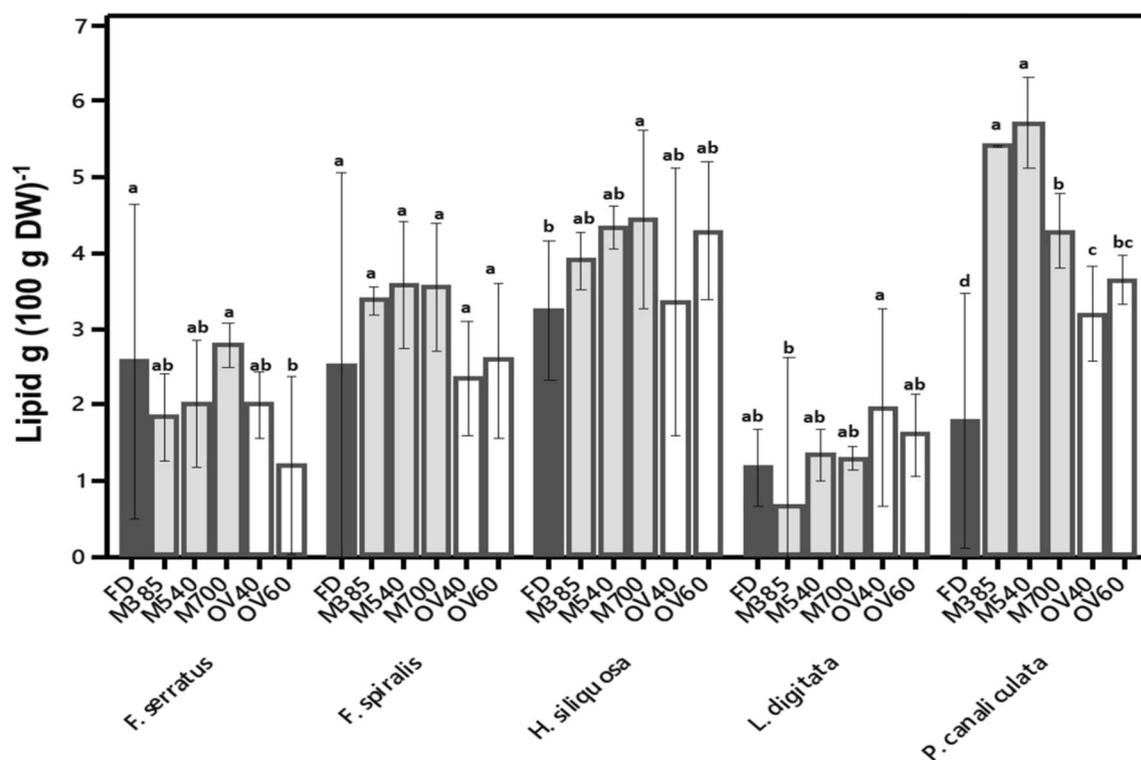


Fig. 7 Lipid contents (g (100 g DW)^{-1}) in *F. serratus*, *F. spiralis*, *H. siliquosa*, *L. digitata* and *P. canaliculata* subjected to freeze-drying (FD—black shading), oven-drying (no shading) at 40 °C (OV40) and 60 °C (OV60) and microwave-drying (grey shading) at 385 W (M385),

540 W (M540) and 700 W (M700). All data points are means of triplicate analyses (independent analytical replicates) and error bars indicate standard deviation. Bars with different letters are significantly different ($P \leq 0.05$; Tukey)

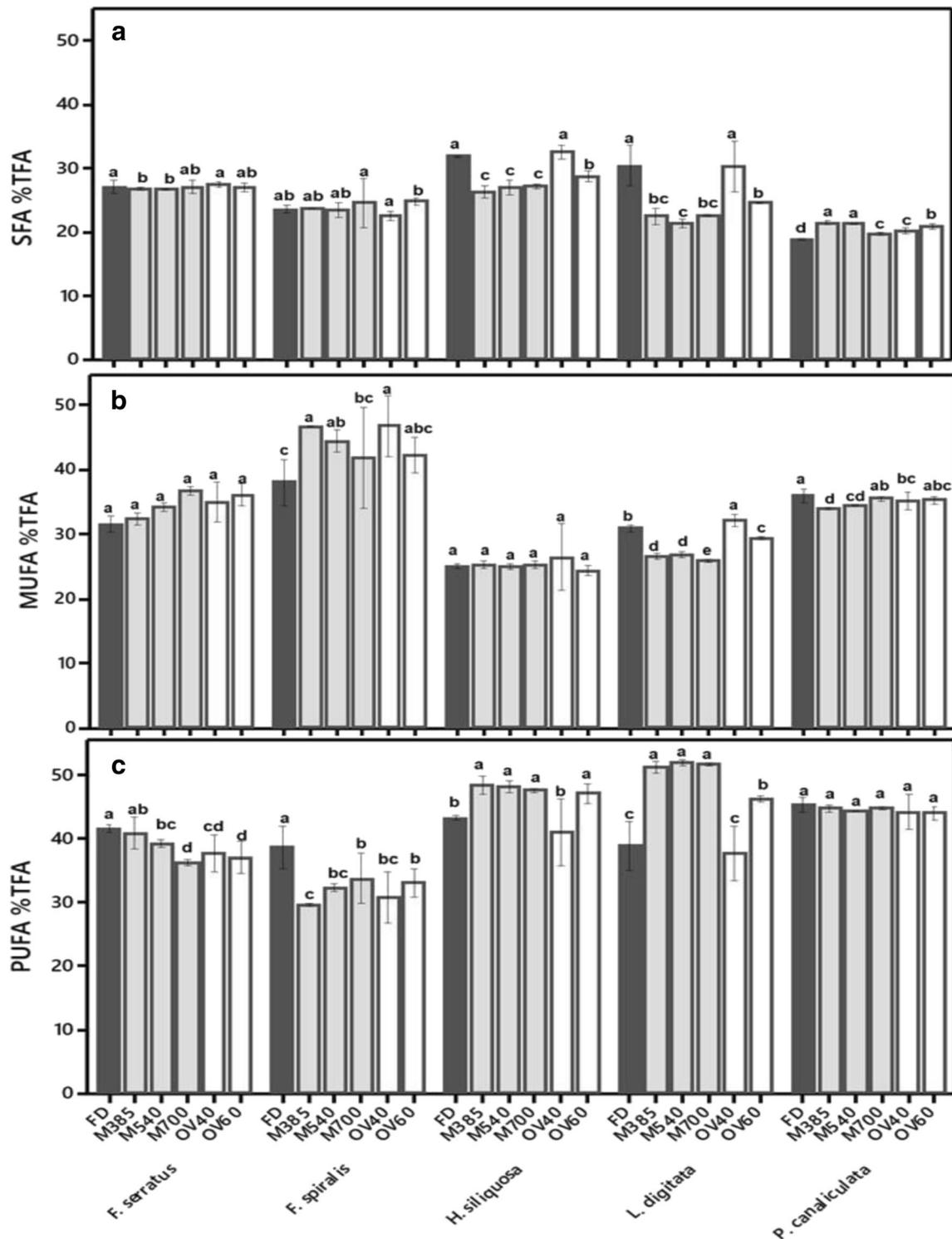


Fig. 8 Fatty acid contents in *F. serratus*, *F. spiralis*, *H. siliquosa*, *L. digitata* and *P. canaliculata* subjected to freeze-drying (FD—black shading), oven-drying (no shading) at 40 °C (OV40) and 60 °C (OV60) and microwave-drying (grey shading) at 385 W (M385), 540 W (M540) and 700 W (M700). (a) Total saturated fatty acid (SFA) (in %TFA); (b) total monounsaturated fatty acid (MUFA) (in %TFA); (c) total

polyunsaturated fatty acid (PUFA) (in %TFA). All data points are means of triplicate analyses (independent analytical replicates) and error bars indicate standard deviation. Bars with different letters are significantly different ($P \leq 0.05$; Tukey). Statistical data are displayed in Table A.4 in the supplementary material

Discussion

Four of the five edible seaweed species studied here are listed in the European Food Safety Authority (EFSA) EU novel foods catalogue, the only exception being *H. siliquosa*, which was included in this study based on previous work that identified the seaweed as an excellent source of phenolic compounds and having strong antioxidant potential (Mutton 2012). In the broadest sense, previous studies have demonstrated the presence of nutritionally important molecules in seaweed, including proteins and amino acids, vitamins, polysaccharides, lipids and fatty acids, polyphenols, carotenoids and other bioactive compounds and minerals (including iodine and other essential elements). Many of these may have therapeutic benefits (acting as antioxidants, antivirals, antihypertensives, anticoagulants, anti-inflammatories, anticancer compounds and thyroid stimulants or having antifungal properties) (Ganesan et al. 2008; Holdt and Kraan 2011; Mohamed et al. 2012). However, many of these potentially beneficial chemical components are also heat-labile—and may be lost or depleted when seaweed is processed—hindering the effective value of certain compounds (Sappati and Nayak 2018). As such, the importance of drying treatment used during processing (on nutritionally important components in seaweed) has been highlighted in several studies. For instance, Chan et al. (1997) considered freeze-drying, oven-drying and sun-drying and the effects on amino acids, fatty acid, mineral and vitamin C in *Sargassum hemiphyllum* (brown seaweed). They showed that sun-drying and freeze-drying reduced the loss of certain important chemical components in comparison with oven-drying. In another study (Stévant et al. 2018) using *Saccharina latissima*, which considered freeze-drying and convective air-drying at 25, 40 and 70 °C, no effects due to drying were observed on the chemical components studied, with the exception of iodine which was lower in freeze-dried batches. Recently, the study of Silva et al. (2019) compared chemical contents in oven-dried (25, 40 and 60 °C) *Ulva rigida*, *Gracilaria* sp. and *Fucus vesiculosus* versus fresh seaweed, and showed that the extraction of polyphenols and their activities is influenced by the drying treatment used.

In this study, drying treatment has been shown to influence the retention of and/or cause depletion in nutritionally important compounds in what would be final seaweed products.

The TPC, TFC and antioxidant activity (TAC and DPPH inhibition) data presented here show that elevated temperature treatments often resulted in products with depleted antioxidant activity and content. This finding agrees with the study of Wong and Chikeung Cheung (2001), wherein higher levels of phenolics were found in freeze-dried batches of three *Sargassum* species when compared with oven-dried batches. Similar results were reported in the study of Stramarkou et al. (2017) regarding the microalgae *Chlorella vulgaris*. Here,

differences in antioxidant activity for all species showed inconsistent effects due to drying treatment. For example, for *F. serratus* and *F. spiralis*, the freeze-dried batches had higher TPC and TFC, and stronger antioxidant activity than the oven-dried (at 60 °C) batches. A similar pattern occurred for *H. siliquosa* with the exception of the TFC, which showed no effect due to drying treatment. In contrast, *L. digitata* did not show appreciable differences in measured antioxidant parameters as a result of different drying conditions. These variations in antioxidant parameters exhibited by the processed seaweeds suggest that drying treatment employed would have a significant influence on either the content or extractability of potential antioxidant compounds (Croces et al. 2016). However, no single drying treatment can be identified as being optimal for all species, possibly due to differences in their cell walls and their physiology (Cox et al. 2012).

In terms of soluble protein levels, relatively low values for all species studied were noted. This includes *L. digitata*, which in previous studies has been shown to contain high levels of protein, ranging from 4.9 to 8.2% DW (Schiener et al. 2014). This could simply be due to differences in the method of extraction used. Here, the method used was reported by Gressler et al. (2010). Differences in results measured by different methods are likely to result from differences in the polysaccharide matrix or in polyphenols, which some proteins bind to and which vary across seaweed species, influencing the extraction of protein (Harnedy and FitzGerald 2013). Regardless, in terms of drying treatment used here, higher protein was found in freeze-dried batches (when compared to oven- and microwave-dried batches), for all species except *L. digitata* (for which limited variation due to drying treatment was observed). These findings agree with Sappati and Nayak (2018) and their protein results for *S. latissima*, but contradict total protein data in Chan et al. (1997) and Wong and Chikeung Cheung (2001) whose results did not show markable effects on crude protein content. Nevertheless, the results presented here support the assumption that the extractability and accessibility of seaweed protein would be influenced by the method used to dry seaweed (Wong and Chikeung Cheung 2001). It would seem logical that freeze-drying (rather than oven-drying) would be the best way to dry seaweed to ease the extraction of protein in seaweed because high-temperature drying treatments would likely result in the denaturing of protein which could result in the protein being less extractable.

Protein quality is as important as protein level in seaweed (Stévant et al. 2018). The type and quantity of individual amino acids present determine the quality of the protein (Maehre et al. 2014). The amino acid content of seaweeds studied here showed similarly low levels to those described in Stévant et al. (2018). Total amino acids, essential amino acids and non-essential amino acids tended to be higher in low-temperature oven-dried batches compared to the other

treatments, for many of the seaweed species studied. The majority of the amino acids present in these seaweeds were non-essential amino acids; however, reasonable levels of essential amino acids were also present. This is a similar finding to that of Maehre et al. (2014), who showed higher non-essential amino acids than essential amino acids in seaweeds. Again, no consistent variation in amino acid composition with drying treatment was observed. In addition, many differences were minor, which is in agreement with the study of Stévant et al. (2018) who also showed no markable differences between oven-drying and freeze-drying for *S. latissima*. Chan et al. (1997) also showed no effects on amino acid content when considering sun-dried, oven-dried and freeze-dried *Sargassum hemiphyllum*.

Variations in total lipid content as a result of drying treatment employed were observed. This agrees with the study of Neoh et al. (2016) which showed higher lipid content in freeze-dried rather than oven-dried *Kappaphycus alvarezii*, whilst Chan et al. (1997), Wong and Cheung (2001) and Sappati and Nayak (2018) all showed no marked variation in lipid content due to drying treatment. It is worth noting that in this study, microwave-drying (which took < 15 min) often produced samples with higher lipid contents for certain species. This could be because of the short duration needed for microwave-drying. Short-duration drying procedures are assumed to overcome issues related to long-term exposure to light and/or high temperatures (which occur during oven-drying and freeze-drying), which can result in the oxidation of lipids. The advantage of microwave-drying in vegetable and fruit processing has been previously highlighted, as, again, this helps prevent quality degradation due to long-term air exposure (Zhang et al. 2006). However, microwave-drying can also have its drawbacks—i.e., related to uneven drying and rapid temperature changes. These can be counteracted (at least in part) by ensuring uniform movement of the product in the microwave oven (i.e., by using a rotating tray); by optimising/controlling power inputs; and by combining microwave-drying with other drying techniques (such as vacuum ovens or freeze-drying; Zhang et al. (2010)).

The lipid content of seaweed is generally low (Mabeau and Fleurence 1993). But, the quality of those lipids due to the presence of essential fatty acids, such as omega 3 and omega 6 (which are not synthesized de novo in humans and are also limited in terrestrial plants), makes seaweed a potential source of essential fatty acids for humans (Monroig et al. 2013). The results here showed that the choice of drying treatment impacted the levels of fatty acids in the seaweeds studied. However, again, no consistent pattern of variation with drying treatment was observed. For example, *H. siliquosa* and *L. digitata* showed higher SFA in the FD than OV60 and *F. serratus* and *F. spiralis* did not show any effect due to drying treatment. Also, the PUFA content in *L. digitata* was higher in all the microwave-dried batches, but for

P. canaliculata, PUFA levels showed no marked effect due to drying treatment. These findings agree with those of Hamid et al. (2018) who also profiled metabolites present in three brown seaweeds and identified changes in profiles due to drying treatment. Species-specific variation in lipids as a result of different drying methods points to the uniqueness and diversity of specific traits associated with marine algae—which exhibit high species variation in cell and tissue structure (wherein, the majority of the phytochemicals are stored; Hurd et al. 2014).

More broadly, the efficient lysis of cells and cellular structures, which aids the release of phytochemicals during extraction, can depend on the drying procedure used (Cox et al. 2012). Effective drying requires sufficient energy to remove water but should ideally not hinder the extractability or diminish the amount of nutritionally important chemical species present in a final product. However, the effect of any drying procedure on the quantity/extractability of nutritionally important chemical species in a final dried product is, at least to some extent, also species specific. This adds an extra layer of complexity when determining the optimal techniques to use, but is also not surprising as the phytochemical profile of individual seaweed species is highly variable and unique. Clearly, drying treatment has the potential to enhance the level of certain chemicals—through the degradation of certain biomolecules into simpler compounds—or cause undesirable degradation of beneficial compounds. Depending on the chemical and biological properties of these various molecules, both could either enhance or reduce the biological and/or chemical activity of the final product (such as the antioxidant capacity). Therefore, drying treatments to be employed in the future for the increased exploitation of seaweed in large-scale food production (or for biotechnological purposes) will require careful optimisation to ensure that compounds of interest remain both available and undegraded in the final product. Moreover, optimal drying techniques are likely to be different for different species and will depend on the key chemical components of interest in any product to be created.

Conclusion

From the results presented here, it is clear that the choice of drying method can significantly influence the presence of nutritionally important compounds and the antioxidant activity of a final dried seaweed product. Additionally, effects are mostly species specific. Hence, to attain efficient exploitation of important chemical constituents present in seaweed, drying conditions should always be optimised and studied in relation to individual species—taking into consideration the compounds of interest and the potential end-use (i.e., whether the preservation of individual chemical components is more critical than it is for others). The findings here also suggest that

short-duration microwave-assisted drying could be a viable option which may help overcome negative effects caused by long-duration drying (such as contamination and/or undesirable oxidation of certain chemical components). Although there is still a need for further research to explore the utility of microwave-assisted drying, it may offer new opportunities, especially if it could be used in combination with other techniques, such as vacuum oven-drying or freeze-drying. Here, both freeze-drying and low-temperature oven-drying proved to be beneficial in terms of preserving certain chemical components. As such, novel combinations involving microwave-drying, freeze-drying and low-temperature oven-drying may have the potential to limit drying duration and aid the preservation of certain chemicals, thus improving the quality of final seaweed products.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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