CHEMICAL COMPOSITION, ANTIOXIDANT ACTIVITY AND SENSORY EVALUATION OF FIVE DIFFERENT SPECIES OF BROWN EDIBLE SEAWEEDS.

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Highlights

The composition and sensory profile of five seaweeds was evaluated.

Fucus sp. and *Ascophyllum nodosum* showed high antioxidant activities.

Nucleotides in *Fucus v*. were 10 times higher than reported in other foods.

Laminaria was significantly different according to panellists.

Abstract.

The chemical and volatile composition as well as sensory profile of five brown edible seaweeds collected in the United Kingdom, was evaluated. The ash content was 190–280 mg/g, NaCl 35.1–115.1 mg/g, protein 2.9–6.0 g/g, and fat 0.6–5.8 g/g (dry basis). *Fucus vesiculosus, Fucus spiralis*. and *Ascophyllum nodosum* showed higher antioxidant activities (DPPH and FRAP). Nucleotide concentrations were of the same order of magnitude as reported in other foods such as tomatoes or potatoes, except for *Fucus vesiculosus* where levels of nucleotides were 10 times higher. The fatty acids profile was dominated by oleic acid (21.9–41.45 %), followed by myristic (6.63–26.75 %) and palmitic (9.23–16.91 %). Glutamic and aspartic acid (0.15–1.8 mg/g and 0.05–3.1 mg/g) were the most abundant amino acids. Finally, sensory and volatile analyses illustrated that *Laminaria sp.* had the strongest seaweed and seafood-like aroma and taste.

Keywords: Seaweeds, Fatty acids, Amino acids, Nucleotides, Antioxidant activity, Sensory evaluation.

Chemical compounds studied in this article:

Oleic acid (Pubchem CID: 445639); myristic acid (Pubchem CID: 11005); palmitic acid (Pubchem CID: 985); eicosapentaenoic acid (Pubchem CID: 446284); docosahexaenoic acid (Pubchem CID: 445580); glutamic acid (Pubchem CID 611); aspartic acid (Pubchem CID: 424); 1-octen-3-ol (Pubchem CID: 18827); 2,4-heptadienal (Pubchem CID: 20307).

1. Introduction.

Due to their low content of lipid, high concentration of polysaccharides, natural richness in minerals, polyunsaturated fatty acids and vitamins as well as their high content of bioactive molecules, marine algae have, in recent years, received great attention (Gupta & Abu-Ghannam, 2011a,b). Algae are grouped into two main categories; the microalgae, found in both benthic and littoral habitats and also throughout the ocean waters as phytoplankton, and the macroalgae or seaweeds, which occupy the littoral zone, and can be classified as red (*Rhodophyta*), brown (*Phaeophyta*) or green (*Chlorophyta*), depending on their nutrient and chemical composition (Dawczynski, Schubert & Jahreis, 2007; Gupta & Abu-Ghannam, 2011a).

Red and brown algae are mainly used, within the traditional Japanese diet as sushi wrappings, seasonings, condiments and vegetables and can thus constitute between 10% and 25% of food intake of most Japanese people. Although the principal uses of seaweeds in Europe are as a source of phycocolloids (thickening and gelling agents) for various industrial applications, including uses in foods or as feed and fertiliser (Ortiz, Bozzo, Navarrete, Osorio & Rios, 2006; Yaich et al., 2011), consumption of seaweed products has recently increased with currently, approximately 15–20 edible algae species being commonly marketed for consumption. These seaweed varieties differ greatly in their quality, colour, consistency, and nutrient content (Dawczynski et al., 2007; Mišurcová, 2011, Mišurcová, Machů & Orsavová, 2011; Mišurcová, Ambrožová & Samek, 2011). Different authors have pointed out that the chemical composition of seaweeds varies with species, habitats, maturity and environmental conditions (Sanchez-Machado, Lopez-Cervantes & Lopez-Hernandez, 2004; Ortiz et al., 2006).

The European seaweed industry is dominated by Norwegian, French and Irish production, while Spain, Portugal and the UK are small producers and suppliers.

69 Particularly, in the UK, the market for seaweed (therapeutic, biotechnology, bio-fuel
70 seaweeds based, or foods) is mostly imported, whereas there is abundance of growing
71 seaweeds around the islands, with some local producers already harvesting them for
72 commercial purposes. Particularly, in the coast of Scotland there are dozens of different
73 kinds of edible seaweed, being the red seaweed dulse (*Palmaria palmata)*, as well as the
74 brown seaweeds: kelp (*Laminaria sp*.) and different wracks (*Fucus sp*., *Ascophyllum*
75 *nodosum*, *Pelvetia canaliculata*) the most generally harvested (due to their abundance
76 and accessibility).
77 The use of brown seaweeds, as ingredient or as a whole food, has already been reported
78 by numerous authors to be beneficial in different aspects. For instance, as an alternative
79 source of protein, with some brown species having higher protein content than
80 soybeans. Their fat content accounts for 1 to 6 g/100 g dry weight with some varieties,
81 as *Laminaria sp.* generally between 1.5 and 3.3% of dry weight (Fleurence, Gutbier,
82 Mabeau, & Leray, 1994), and some of these species are also characterised by a high
83 level of eicosapentaenoic acid (up to 24% of the total fatty acid fraction) (Fleurence,
84 2004). Antioxidants are also other important metabolites in brown seaweeds including
85 fucoxanthin, polyphloroglucinol, phenolic compounds or bromophenols, that have been
86 isolated from species such as *Fucus* and *Laminaria* (Xu et al., 2004a; 2004b; Gupta &
87 Abu-Ghannam, 2011b; Fleurence et al., 2012)
88 In addition, there are recent projections in the functional effects of seaweeds as means
89 to improve the fibre content and reduce the salt content of food products. This is mainly
90 due to their high content in umami compounds such as nucleotides or some amino acids.
91 The aim of this study was to characterise five different brown edible seaweeds locally
92 produced on the west coast of Scotland (Isle of Bute), UK, in terms of chemical

composition as well as sensory and volatile analyses; this information might be useful to evaluate their use as food ingredients and their potential contribution to the diet.

2. Material and Methods

2.1. Raw material.

Five different species of brown seaweed (*Laminaria digitata, Ascophyllum nodosum, Pelvetia canaliculata, Fucus vesiculosus, and Fucus spiralis*), were obtained from the same supplier and harvested between May and August 2012 in the west coast of Scotland, United Kingdom. The samples were then freeze-dried and separated into two different batches depending on the harvesting time; seaweeds collected in May and June (batch 1), and those collected in July and August (batch 2). Samples were milled in a mechanical grinder for 10 min, to obtain a fine and homogeneous powder before performing the analyses.

2.2. Chemical analyses.

All the chemical analyses were carried out in triplicate on the homogeneous powder.

*2.2.1. Dry matter, ash and NaCl content***.**

The dry matter, ash and sodium chloride content were ascertained according to the Association of Official Analytical Chemists (AOAC, 2000).

2.2.2. Protein.

Total protein was determined by the Kjeldahl method. The protein was calculated using a nitrogen conversion factor of 6.25 (Ortiz et al., 2006; Yaich et al., 2011). Data were expressed as percentage of dry weight.

2.2.3. Extractable fat.

The extractable fat was determined using the Soxhlet extraction method with petroleum ether 40:60 as solvent. (AOAC, 2000).

118 *2.2.4. Fatty acids.*

119 The fatty acid composition was analysed by GC-FID after transesterification to methyl
120 esters (FAMEs) with a mixture BF_3 methanol at 20 ºC according to the IUPAC standard
121 method (IUPAC, 1992, Yaich et al., 2011).

122 Fat (10 mg), hexane (0.2 mL) and BF_3 (0.5 mL) were heated at 70 ºC for 1.5 h. After
123 transesterification, saturated salt solution (0.5 mL, 25 % NaCl), H_2SO_4 (0.2 mL, 10%)
124 and hexane (7 mL) were added to the reaction medium. Analysis of FAMEs was carried
125 out with a Hewlett Packard 6890 GC equipped with an auto sampler, an Agilent 6890
126 Network FID and an Agilent DB-23 (60 m × 0.25 mm, 0.25 µm) capillary column. The
127 oven temperature was programmed from 90 ºC to 240 ºC at 4 ºC/min and the injector
128 and detector temperatures were set at 250 ºC. The carrier gas was helium at 1.0 mL/min
129 constant flow (split ratio 10:1). The software used for data acquisition and processing is
130 6890N. Data Analysis Identification and quantification of FAMEs was accomplished by
131 comparing the retention times of the peaks with those of pure standards (Supelco® 37
132 Component FAME Mix**,** Sigma**)** and analysed under the same conditions. The results
133 were expressed as percentage of individual fatty acids in the lipid fraction.

134 *2.2.5. Antioxidants*

135 Seaweed powder (0.1 g) was mixed with 2.5 mL ethanol (95 %), vortexed for 30 s and
136 stored at -20 ºC overnight. The sample was centrifuged for 10 min at 2000 × g at room
137 temperature under dark conditions and the supernatant was used for analysis.

138 The radical scavenging activity (DPPH), was determined following the modified
139 protocol of Brand-Williams, Cuvelier & Berset (1995). Sample (10 µL) and deionized
140 H_2O (90 µL) were added in a 96-well microtiter plate and the reaction started by adding
141 200 µL of freshly prepared DPPH solution (0.024 g/L DPPH). The absorbance was

142 measured at 515 nm every 4 min for 32 min in total, when the absorbance value
143 remained constant.
144 The reducing power of the samples (FRAP), was determined by the modified protocol
145 described by Benzie & Szeto (1999) and Bub *et al*. (2000), in a 96-well microtiter plate,
146 following a similar procedure as for DPPH. In this case the reaction was started by
147 adding pre-warmed FRAP reagent (200 μL, 37 ºC), the absorbance was determined at a
148 wavelength of 593 nm and the reaction time was 8 min. at 37 ºC.
149 Finally, the total phenolic content (TPC) was determined following the modified
150 protocol of the microplate Folin-Ciocalteu assay (Magalhães, Santos, Segundo, Reis &
151 Lima, 2010). Samples (50 μL, [1:10 v/v]) were added to Na_2CO_3 solution (100 μL, 6%
152 [w/v]). The reaction was started by adding the Folin-Ciocalteu solution (50 μL, [1:25
153 v/v]), and the absorbance determined at 725 nm every 5 min for a total of 30 min, when
154 the absorbance value remained constant.
155 For the DPPH and FRAP assay calibration curves of Trolox (0-1000 mM) were
156 prepared and results were expressed as the number of equivalents of Trolox (mmol eq of
157 Trolox/g dry weight). Gallic acid (0-1000 mM) was used for TPC and results expressed
158 as the number of equivalents of gallic acid (mmol eq of gallic acid/g dry weight of
159 seaweed powder).
160 *2.2.6. Nucleotides*.
161 Nucleotides were extracted using water and hydrochloric acid following centrifugation
162 based on a modified version of the protocol by Oruña-Concha, Methven, Blumenthal,
163 Young & Mottram (2007). Freeze-dried samples (0.3 g) were weighed into 15 mL
164 screw-top vials; distilled water (5 mL) and hydrochloric acid (5 mL, 0.01 N, HCl) were
165 added followed by stirring at 90 ºC for 90 min. The mixture was allowed to stand for

another 20 min and aliquots of the supernatant (1.5 mL) were centrifuged at 8500 × g for 15 min.

The 5'-nucleotides were separated using a Dionex Ultimate 3000 HPLC system attached to a UV-spectrophotometric detector, HPG-3200 pump, and a 10 µL sample loop, using solvent A (KH_2PO_4 0.04 M, pH 5.5) and solvent B (KH_2PO_4 0.5 M, pH 5.5) as a mobile phase. Gradient elution was carried out as follows: 0–15 min 100% A, 15–20 min 100% B, 20–25 min 100% A (initial conditions), 25 min re-equilibration wash with 100% A, at a flow rate of 1 mL/min, using a SphereClone 5 µm SAX 80 Å, LC Column 250 x 4.6 mm (Phenomenex [phenomenex.com]), and UV detection at 254 nm. Each 5'-nucleotide was quantified using a calibration curve of the pure 5'-nucleotide (5'-guanosine monophosphate (GMP), 5'-inosine monophosphate (IMP) 5-adenosine monophosphate (AMP) and uridine monophosphate, (UMP)). Recovery rates were determined by standard addition methodology.

2.2.7. Amino acids.

An aliquot of the extract used for nucleotides analyses (100 µL) was derivatised using the EZ-Faast amino acid kit (Phenomenex, Torrance, CA). GC-MS analysis were carried out using an 6890 GC coupled to a 5973 MSD instrument (Agilent, Palo Alto, CA) as described by (Elmore, Koutsidis, Dodson, Mottram & Wedzicha, 2005). Norvaline was used as internal standard and calibration curves were used for the quantification of the amino acids.

2.2.8. Volatiles analysis

GC-MS analysis was performed using an Agilent 7890A gas chromatograph equipped with a CPWAX capillary column (60m × 0.25mm i.d. × 0.25µm FT) and coupled to a BenchToF Time of Flight Mass Spectrometer (Almsco, UK) and a CTC CombiPal autosampler (CTC Analytics AG, Zwingen, Switzerland). HS-SPME was performed on

191 the aqueous extracts used for sensory evaluation (200 µL) in 2mL of saturated NaCl
192 solution. The samples were incubated at 40°C for 40 min followed by a 1 min extraction
193 using a CAR/PDMS/DVB SPME fibre and desorption at 260 °C for 10 min. The oven
194 temperature was programmed as follows: initial temperature 40ºC (held for 5min), 40-
195 200ºC at 4ºC/min, then to 250ºC at 8ºC/min, held for 5 min. Helium was used as the
196 carrier gas at a flow rate of 1mL/min.
197 The volatile compounds were identified by comparing their mass spectra (m/z values of
198 the most important ions) with spectral data from the National Institute of Standards and
199 Technology 2002 library as well as retention indices published in the literature
200 (pherobase.com) Relative retention indices were determined by injection into the
201 column of a solution containing the homogenous series of normal alkanes (C_7–C_{30}; by
202 Sigma-Aldrich) in the same temperature programmed run, as described above.
203 Quantification of selected compounds was carried out using external calibration curves.
204 *2.2.9. Sensory evaluation.*
205 Aqueous extracts in mineral water (1%, w/w) were heated at 70 ºC for 30 min and
206 filtered before sensory evaluation. This temperature was chosen as the enzymic
207 degradation processes which break down the RNA into 5'-nucleotides are pH and
208 temperature dependant; and as temperature increases during heating of the samples,
209 nuclease activity increases to around 65-75 ºC (Solms & Wyler, 1979; Yang, Lin, &
210 Mau, 2001). Extracts were analysed by conventional sensory profiling, using a non-
211 trained panel (n=21; 9 female, 12 male). The size of the panel used could be considered
212 small for the general requirements of a conventional sensory profile; nevertheless, for
213 the aim of this sensory study, which was to get a general idea of the perception of the
214 attributes by consumers that would not be very familiar with that kind of product, the
215 use of that sort of panel would be enough according to some previous studies

(Clapperton & Piggott 1979; Delahunty, McCord, O'Neille & Morissey, 1997; Husson, Le Dien & Pagés, 2001; Husson & Pagés, 2003). The sensory attributes studied, which had been previously described by 4 assessors, were: honey-like odour, herbal odour, seaweed-like odour, seafood-like taste, saltiness, astringency, bitterness, green tea-like taste, and salmon-like taste. 10 mL of each seaweed extract at room temperature was served to each panellist. Continuous non-structured scales were used for evaluation. The left side of the scale corresponded to the lowest intensity (value 0) and the right side to the highest intensity (value 10). Each panellist rinsed their mouth with mineral water and ate a piece of plain cracker between samples.

2.3. Statistics

Analysis of variance (ANOVA) and the Friedman test (p-value < 0.05) were carried out using SPSS to estimate the differences in composition of the seaweed varieties investigated in this study.

Principal Component Analysis, PCA, (SPSS) was also applied to differentiate the varieties of seaweeds based on their chemical composition and volatile compound profile.

3. Results and discussion

3.1. Dry weight, contents of ash, NaCl, protein and extractable fat.

Table 1 illustrates the chemical composition of the five different varieties of seaweed depending on the time of harvest. Significant differences ($p<0.05$) were found in their composition depending on season (batch) and also on the species. In general terms, the values obtained were of the same order of magnitude as those reported by other authors for brown seaweeds (Ito & Kanji, 1989; Ortiz et al., 2006; Rioux, Turgeon, & Beaulieu, 2009; Gómez-Ordóñez, Jiménez-Escrig & Rupérez, 2010). It is important to point out

241 the high salt levels (NaCl) presented by *F. spiralis* and *L. digitata.* No inter-species or
242 inter-batch differences were found in the protein content for these two seaweeds, their
243 values being similar to those reported by Yaich et al., 2011 (8.46% dry weight) and
244 Ortiz et al., 2006; (10 % dry weight), but slightly lower than those reported by other
245 authors for brown seaweeds (Rioux, Turgeon, & Beaulieu, 2009; Gómez-Ordóñez et al.,
246 2010). These differences might be expected as variations in the protein content of
247 seaweeds can be attributed to species differences and seasonal effects (Fleurence, 1999;
248 Yaich et al., 2011). Extractable lipid varied among the different species, but was of the
249 same order of magnitude as the contents reported by other authors, such as Ito & Kanji,
250 (1989) (0.1- 4.9 % dry weight) or Gómez-Ordóñez et al., 2010 (0.94-5.97 % dry
251 weight). *F. vesiculosus* and *P. canaliculata* where the two species with the highest
252 extractable fat content. Differences observed, between batches or species, could be
253 attributed to factors such as climate, geographical origin of the seaweed and the method
254 used to extract oil.

255 ***3.2. Antioxidant activity***

256 The antioxidant activity of the ethanolic extracts of the seaweed samples was analysed
257 by two different methods to accurately reflect all the antioxidants in the samples (Table
258 1). The FRAP reagent can react with iron (II) and thiol groups (Benzie & Szeto, 1999),
259 while DPPH is expected to react with organic radicals (Chandrasekar, Madhusudhana,
260 Ramakrishna & Diwan, 2006). The values for the total phenolic content are also
261 presented in Table 1 (mmol equivalents of gallic acid/g dry weight). The estimation of
262 the antioxidant potential using different methods enables a better understanding of the
263 mechanism(s) of antioxidative action of the seaweed extracts.

264

Table 1.
Composition of the seaweed samples: moisture (x^w %), ash (% dry weight), NaCl (mg / g dry weight), protein (g / g dry weight) and fat content (g / g dry weight), antioxidant activity (DPPH and FRAP mET/100g of dry weight), total phenolic content (mEG /100g of dry weight), fatty acids composition (g/100g of total fat), and homogeneous groups obtained from the statistical analysis for the different species of seaweeds and the different batches used (n=3).

		Batch	*Laminaria digitata.*	*Aschophyllum nodosum.*	*Pelvetia canaliculata.*	*Fucus vesiculosus.*	*Fucus spiralis.*
Fresh	x^w	1	81.0 ± 0.5	69.0 ± 0.2	64.6 ± 3.2	60.0 ± 0.5	76.7 ± 0.5
		2	81.0 ± 0.5	68.1 ± 2.3	66.4 ± 5.4	58.2 ± 3.0	74.3 ± 0.6
Freeze dried	Ash	1	21.0 ± 0.2 (a)	19.0 ± 0.2 (a)	21.0 ± 0.2 (a)	21.0 ± 0.2 (a)	25.0 ± 0.2 (c)
		2	28.0 ± 0.2 (d)	22.0 ± 0.2 (b)	22.0 ± 0.2 (b)	19.0 ± 0.2 (a)	26.5 ± 0.7 (c)
	NaCl	1	91.7 ± 1.0 (c)	41.8 ± 0.2 (b)	35.1 ± 0.6 (a)	51.2 ± 0.3 (b)	94.6 ± 1.7 (c)
		2	115.1 ± 0.2 (d)	61.1 ± 0.4 (b)	51.3 ± 0.7 (b)	49.8 ± 4.0 (b)	93.1 ± 4.3 (c)
	Protein	1	5.79 ± 0.08 (b)	5.24 ± 0.01 (b)	7.26 ± 0.30 (c)	5.80 ± 0.17 (b)	5.89 ± 0.30 (b)
		2	5.25 ± 0.20 (b)	4.25 ± 0.04 (b)	4.08 ± 0.28 (b)	2.95 ± 0.66 (a)	5.99 ± 0.12 (b)
	fat	1	0.57 ± 0.18 (a)	1.82 ± 0.31 (b)	5.06 ± 0.16 (d)	3.95 ± 0.17 (c)	2.51 ± 0.31 (b)
		2	0.67 ± 0.15 (a)	2.89 ± 0.02 (b)	5.81 ± 0.21 (d)	4.64 ± 0.23 (c)	1.99 ± 0.06 (b)
Antioxidant activity	DPPH[a]	1	5.1± 1.7 (a)	50.2 ± 3.5 (d)	37.4 ± 3.9 (c)	40.4 ± 2.3 (c)	40.0 ± 2.8 (c)
		2	15.1 ± 1.4 (b)	50.3 ± 6.0 (d)	41.8 ± 1.4 (c)	50.7 ± 3.7 (d)	54.5 ± 0.4 (d)
	FRAP[a]	1	–	21.1 ± 0.8 (d)	10.2 ± 0.7 (b)	55.0 ± 2.3 (e)	19.1 ± 1.1 (c)
		2	–	25.8 ± 1.2 (d)	11.3 ± 0.3 (b)	49.7 ± 1.6 (e)	18.8 ± 0.7 (c
	TPC[b]	1	0.04 ± 0.02 (a)	1.69 ± 0.03 (b)	1.68 ± 0.20 (bc)	2.31 ± 0.02 (c)	1.15 ± 0.0 6 (b)
		2	0.03 ± 0.02 (a)	2.11 ± 0.06 (c)	0.91 ± 0.02 (b)	2.53 ± 0.04 (c)	1.44 ± 0.05 (b)
Fatty acids	C10	1	5.9 ± 0.4 (a)	4.5 ± 0.3 (a)	4.0 ± 1.3 (a)	2.8 ± 0.4 (a)	3.2 ± 1.0 (a)
		2	17.6 ± 3.5 (b)	10.4 ± 2.3 (b)	7.8 ± 2.8 (ab)	18.8 ± 0.2 (b)	12.9 ± 1.2 (b)
	C14	1	9.9 ± 0.4 (ab)	10.6 ± 1.1 (ab)	12.0 ± 2.5 (b)	13.9 ± 0.9 (b)	15.5 ± 0.6 (b)
		2	10.3 ± 1.2 (ab)	13.1 ± 0.2 (b)	10.2 ± 0.4 (ab)	7.5 ± 0.4 (a)	11.3 ± 0.3 (b)
	C16	1	18.8 ± 0.5 (c)	12.7 ± 2.8 (ab)	13.8 ± 1.1 (b)	12.1 ± 0.2 (ab)	14.4 ± 1.1 (b)
		2	16.3 ± 2.0 (c)	11.8 ± 0.9 (a)	10.0 ± 0.4 (a)	9.6 ± 0.2 (a)	13.6 ± 0.3 (b)
	C18:1	1	28.8 ± 0.8 (b)	44.9 ± 7.5 (c)	46.0 ± 0.6 (c)	46.9 ± 0.3 (c)	33.1 ± 0.7 (b)
		2	16.7 ± 2.6 (a)	46.5 ± 0.2 (c)	46.5 ± 3.6 (c)	31.9 ± 2.5 (b)	33.3 ± 1.1 (b)
	C18:2	1	4.8 ± 0.2 (a)	7.0 ± 1.1 (a)	12.0 ± 0.4 (d)	10.0 ± 0.2 (bc)	11.7 ± 0.2 (cd)
		2	8.4 ± 1.1 (ab)	9.1 ± 1.8 (b)	11.1 ± 0.2 (c)	7.5 ± 0.7 (a)	8.9 ± 0.4 (ab)
	C18:3	1	2.3 ± 0.2 (b)	1.4 ± 0.2 (a)	3.1 ± 0.2 (b)	3.4 ± 0.2 (b)	3.8 ± 0.2 (b)
		2	5.4 ± 0.4 (c)	–	2.1 ± 0.6 (b)	–	2.3 ± 0.3 (b)
	C20:5	1	5.0 ± 0.2 (ab)	5.9 ± 1.2 (ab)	8.3 ± 0.2 (b)	6.7 ± 0.2 (ab)	6.8 ± 0.2 (ab)
		2	4.8 ± 0.2 (ab)	5.9 ± 0.2 (ab)	5.8 ± 0.2 (ab)	4.5 ± 0.2 (a)	4.0 ± 0.2 (a)
	C22:6	1	2.8 ± 0.1 (a)	2.2 ± 0.2 (a)	2.5 ± 0.2 (a)	2.3 ± 0.2 (a)	3.3 ± 0.2 (a)
		2	7.5 ± 0.2 (b)	–	0.7 ± 0.2 (a)	–	2.2 ± 0.3 (a)

a, b, c and d: homogeneous groups obtained from the statistical analysis (ANOVA), for the different species of seaweeds and the different batches used (n=3).
[a] (mmol equivalents of Trolox/ g DW); [b] (mmol equivalents of Gallic Acid / g DW)

There were differences between the seaweeds species in terms of their antioxidant activity values with *Fucus sp*. and *Ascophyllum nodosum* being the ones with the highest values (40-50 mmol Trolox/g dry weight [DPPH], 21-55 mmol Trolox/g dry weight [FRAP]). These values are in the same order of magnitude that those reported previously (Díaz-Rubio, Pérez-Jiménez & Saura-Calixto, 2009). *Fucus sp.* and *Aschophylum sp*. were also found to be the species with the highest antioxidant values among different brown seaweed species by Wang, Jónsdóttir & Ólafsdóttir (2009). In general terms, DPPH and FRAP values followed the same pattern in the seaweed

284 samples but DPPH values were slightly higher than FRAP values. The DPPH method
285 measures free radical-scavenging ability and higher values might be due to higher levels
286 of phenolic compounds. Catechin, epigallocatechin, phlorotaninns and fucoxantines
287 have all been reported in brown seaweed (Langley-Evans, 2000; Jaime, Pulido & Saura-
288 Calixto, 2001; Kuda, Tsunekawa, Goto & Araki, 2005; Meenakshi, Umayaparvathi,
289 Arumugam & Balasubramanian, 2011; Chakraborty, Praveen, Vijayan, & Rao, 2013).
290 The DPPH data reported here may also indicate the presence of secondary metabolites
291 with antioxidant activity, such as phlorotannins and fucoxanthin, which have previously
292 been reported to be active compounds with antioxidant properties in brown seaweeds
293 (Meenakshi et al., 2011). The antioxidant values exhibited in the present study may be
294 due to the presence of such compounds or any other potential antioxidants with centre/s
295 of unsaturation.
296 Regarding the FRAP assay, the reducing abilities of chemical compounds, are generally
297 dependent on the presence of reductones, which have been shown to impart antioxidant
298 action by breaking the free radical chain reaction. The presence of antioxidants
299 (reductants) in the samples leads to reduction of the Fe^{3+}/ferricyanide complex to its
300 Fe^{2+} form. The results obtained in the present study are in accordance with earlier
301 reports, where it was suggested that brown seaweeds show potential reducing abilities.
302 The reduced form of iron (Fe^{2+}) can stimulate and accelerate lipid peroxidation by
303 decomposing lipid hydroperoxides into peroxyl and alkoxyl radicals, that can
304 themselves, abstract hydrogen and perpetuate the chain reaction of lipid peroxidation.
305 As a result, chelators of Fe^{2+} ion can be considered as potential inhibitors of lipid
306 peroxidation. However, the chelating abilities of the samples in the current study may
307 also be due to the presence of different types of polysaccharides. Molecules with
308 hydroxyl, sulfhydryl, carbonyl, and phosphate groups have been reported to possess

309 favourable structure-function configuration resulting in Fe^{2+} chelating abilities.
310 Compounds such as phenolic acids, the flavonoid, quercetin, and phenolic glycosides
311 are known to chelate transition metal ions like Fe^{2+} iron. These active compounds might
312 have a synergistic effect, playing an important role in antioxidant activity by the
313 inhibition of oxidation and chelating effects (Rajauria, Jaiswal, Abu-Ghannam & Gupta,
314 2010; Cho, Lee, Kang, Won & You, 2011; Costa, Gonçalves, Andrade, Valentão &
315 Romano, 2011).

316 ***3.3. Fatty acid composition***

317 The fatty acid composition of the two batches of seaweed samples is given in Table 1.
318 The most abundant fatty acids were oleic acid $C_{18:1}$ (21.9 to 41.45 %), myristic $C_{14:0}$
319 (6.63 to 26.75 %) and palmitic $C_{16:0}$ (9.23 to 16.91 %) while the results are comparable
320 to those presented by other authors for green and brown seaweeds. Ortiz et al., (2006)
321 reported that oleic acid was the most abundant monounsaturated fatty acid in samples of
322 brown seaweeds collected from the coastal area of Northern Chile while, palmitic was
323 found to be the most abundant fatty acid by other authors (16 to 63% of total fatty acids)
324 (Sanchez-Machado et al., 2004; Yaich et al., 2011). In the present study, the percentages
325 of fatty acids differed among the species of seaweeds; *Laminar*ia, contained the lowest
326 percentage of myristic (10.1 ± 0.03 %) and oleic (22.7 ± 8.6 %) but the highest
327 percentage of palmitic (17.5 ± 1.8 %) contrary to other species such as *Fucus v*. or
328 *Pelvetia c*. which contained low percentages in palmitic (10.8 ± 1.6 and 11.3 ± 1.8 %
329 respectively) but higher contents of oleic (39.3 ± 1.5 and 46.3 ± 0.4 % respectively).
330 Finally, there were no significant differences in the percentages of the long-chain
331 omega-3 fatty acids (EPA: $C_{20:5}$ eicosapentanioic acid, and DHA: $C_{22:6}$ docosahexanoic
332 acid), among the different seaweeds species, although there were seasonal differences in
333 EPA content for *P. canaliculata* and *F. spiralis*. Variations in fatty acid contents are

attributable both to environmental and genetic differences. Although seaweeds are not a conventional source of energy (their total lipid content is low compared to other foods), their polyunsaturated fatty acid contents can be as high as those of terrestrial vegetables (Sanchez-Machado et al., 2004).

3.4. Free amino acids, nucleotides and umami contribution

The free amino acid composition (mg/ g of dry weight) is illustrated in Table 2. It is important to point out, the high alanine content in the seaweeds collected in July and August of *L. digitata* (4.1 ± 0.2 mg/ g of dry weight) compared to those collected earlier for the same species, but also compared to the others. Glutamic acid was particularly high in *P. canaliculata* and *F. spiralis*, while aspartic acid was the highest amino acid in *F. spiralis*.

Table 2.
Quantities of 5'ribonucleotides, amino acids and Equivalent Umami Concentration found in the different species of seaweeds and the different batches used (n=3).

	Batch	*Laminaria digitata.*	*Aschophyllum nodosum.*	*Pelvetia canaliculata.*	*Fucus vesiculosus.*	*Fucus spiralis.*
5'Nucleotides[a]						
UMP	1	142.1 ± 6.4	97.5 ± 13.7	167.4 ± 17.9	1754.9 ± 119.7	259.0 ± 38.3
	2	81.7 ± 4.7	-	294.7 ± 10.0	1946.9 ± 100.5	104.0 ± 10.0
IMP	1	-	-	-	1229.3 ± 109.5	15.5 ± 0.6
	2				1390.0 ± 87.7	11.3 ± 0.3
GMP	1	69.7 ± 26.7	96.2 ± 28.0	87.3 ± 6.9	3873.0 ± 295.0	364.3 ± 13.2
	2	110.4 ± 0.7	187.5 ± 51.2	136.4 ± -	3908.5 ± 308.9	235.9 ± 10.8
AMP	1	-	55.7 ± 4.1	-	74.3 ± 0.2	125.8 ± 9.7
	2		-		-	-
Amino acids[b]						
GLU[c]	1	0.15 ± 0.03	0.72 ± 0.16	1.02 ± 0.09	0.43 ± 0.13	1.65 ± 0.13
	2	0.61 ± 0.26	0.47 ± 0.12	1.32 ± 0.25	0.54 ± 0.25	1.25 ± 0.29
ASP[c]	1	0.05 ± 0.02	1.06 ± 0.13	0.22 ± 0.02	0.25 ± 0.06	2.75 ± 0.12
	2	0.23 ± 0.06	1.44 ± 0.27	0.21 ± 0.07	0.71 ± 0.08	3.09 ± 0.47
Alanine	1	0.72 ± 0.07	0.70 ± 0.02	0.31 ± 0.02	0.35 ± 0.02	2.62 ± 0.09
	2	4.13 ± 0.16	0.39 ± 0.02	1.01 ± 0.02	0.44 ± 0.02	1.37 ± 0.02
Proline	1	0.005 ± 0.002	0.011 ± 0.002	0.010 ± 0.002	0.017 ± 0.002	0.058 ± 0.008
	2	0.025 ± 0.003	0.014 ± 0.002	0.017 ± 0.002	0.023 ± 0.002	0.040 ± 0.003
Asparagine	1	-	0.154 ± 0.019	0.075 ± 0.013	0.483 ± 0.005	0.230 ± 0.004
	2		0.069 ± 0.002	0.051 ± 0.006	0.152 ± 0.018	0.274 ± 0.046
EUC[d]	1	0.31 ± 0.05	2.29 ± 0.06	1.75 ± 0.31	55.44 ± 12.61	21.05 ± 6.41
	2	1.81 ± 0.48	3.03 ± 0.09	3.04 ± 0.41	74.44 ± 27.01	13.83 ± 2.76

[a] µg/ g of dry weight.
[b] mg/ g of dry weight.
[c] Umami amino acids (Glutamic acid and Aspartic acid).
[d] g MSG/ 100 g.

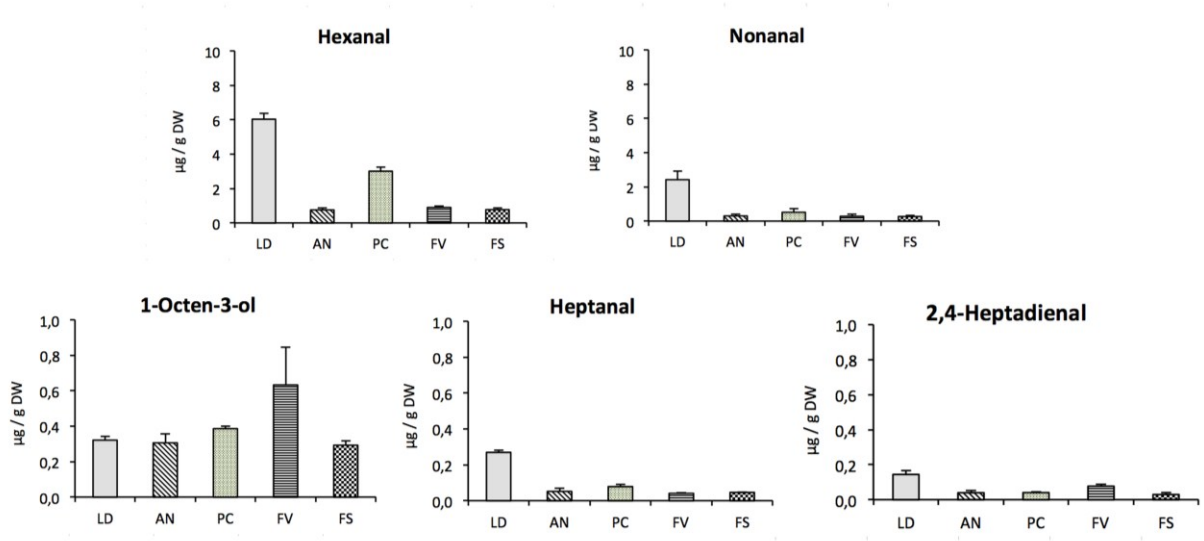
354 Similar results were found by other authors such as Yaich et al. (2011) and Dawczynski
355 et al. (2007) who found that aspartic acid and glutamic acid constituted, a substantial
356 amount of the total amino acids (26 %) for green and brown seaweeds. The contents of
357 glutamic and aspartic acid were of the same order of magnitude as those found for other
358 foods such as tomatoes or potatoes (Morris, Ross, Ducreux, Bradshaw, Bryan & Taylor,
359 2007; Oruña-Concha et al., 2007; Coulier, Bas, Hekman, Van der Werff, Burgering &
360 Thissen, 2011;), but in considerably lower amounts than have been found in some
361 species of mushrooms (40 mg / g dry weight) (Beluhan & Ranogajec, 2011).
362 The nucleotide composition (µg/ g of dry weight) for the five seaweeds samples is given
363 in Table 2. These values ranged from 0.20 ± 0.02 to 364.3 ± 13.2 µg/ g of dry weight,
364 and were of the same order of magnitude as reported in other foods such as tomatoes,
365 potatoes or some varieties of mushrooms (60 to 300 µg / g of dry weight) (Morris et al.,
366 2007; Oruña-Concha et al., 2007; Cho, Choi & Kim, 2010). Nevertheless, it is important
367 to highlight that the amount of the different nucleotides was found to be ten times
368 higher for *Fucus v*., compared with the other seaweeds, which is similar to the
369 concentrations found by Beluhan & Ranogajec, (2011) in some species of mushrooms.
370 It has previously been suggested that four 5'-nucleotides (5'-AMP, 5'-IMP, 5'-GMP,
371 and 5'-XMP [xanthosine monophosphate]) contribute to umami taste in mushrooms;
372 and the umami taste would synergistically increase by the combination of umami amino
373 acids and the umami 5'-nucleotides (Yamaguchi, Yoshikawa, Ikeda & Ninomiya,
374 1971). The EUC value of 100% indicates that the umami intensity of sample per g of
375 dry matter is equivalent to the umami intensity of 1 g of MSG (monosodium glutamate-
376 like). The EUC values of the different seaweed species are illustrated in Table 2, and
377 they varied widely, ranging from 0.31 ± 0.05 in *Laminaria d*. (batch 1) to 74.5 ± 27.0 %
378 in *Fucus v*. (batch 2) The high levels of aspartic and glutamic acids, in combination with

379 the nucleotides content might be responsible for the characteristic flavour and taste of
380 seaweeds.

381 ***3.5. Volatiles analysis***

382 A total of 23 compounds were detected and identified in the aqueous extracts of the 5
383 seaweeds. Volatile compounds identified in the different seaweed samples are presented
384 in Table 3 and can be classified as aldehydes, alcohols, esters, ketones, acids and
385 aromatic compounds. Five key compounds, (hexanal, heptanal, nonanal, 1-octen-3-ol
386 and 2,4-heptadienal), which have previously been described as giving rise to fishy notes
387 (Ganeko, et al., 2008; Giri, Osako & Ohshima, 2010) were studied in more detail. They
388 were quantified using external calibration curves and the Friedman test was applied to
389 study any differences in their concentrations between the aqueous seaweed extracts
390 (Fig. 1).

391



392

393 **Fig. 1.** Concentration of the most relevant seafood volatile compounds in the aqueous extracts used for
394 sensory evaluation (µg/g DM), quantified using external calibration curves (LD: *Laminaria digitata,* AN:
395 *Ascophyllum nodosum,* PC: *Pelvetia canaliculata,* FV: *Fucus vesiculosus, and* FS: *Fucus spiralis*).
396

397

398 **Table 3.**
399 Retention time, retention index and odour descriptors of volatile compounds found in the different species of
400 seaweeds and the different batches used (n=3).

	RT	RI	Identification	Odour description
Aldehydes				
hexanal	16.551	1080	MS, RI Std	Fishy, grass[A,B,C]
heptanal	21.604	1170	MS, RI Std	Dry fish[A,D] Citrus fruit [B,C,D], Green, Fatty, Pesticide, Solvent, Smoky, Rancid, Fruity[D]
octanal	26.057	1286	MS, RI Std	Fatty, pungent[A], fatty-orange odour[B,C], Lemon, Stew-like, Rancid, Soapy, Citrus, Green, Flower, Fruit, Orange[D]
2-heptenal	27.426	1326	MS, RI Std	Pungent green, somewhat fatty aroma[C]
nonanal	30.022	1404	MS, RI Std	Green, fatty [A,B,C,D] Floral, Waxy, Sweet, Melon, Soapy, Lavender, Citrus fruit[D]
2-octenal	31.328	1512	MS, RI Std	Aromatic, oxidized oil-like[B] ,Fatty, Nutty, Burdock-like, Sweet, Sour, Waxy, Green, Burnt, Mushroom[D]
2,4-heptadienal	32.529	1531	MS, RI Std	Fatty, fishy[A,C], aromatic, oxidized oil-like[B]
Alcohols				
1-penten-3-ol	20.321	1148	MS, RI Std	Burnt, meaty[A,] paint like chemical like[B] grassy-green[C]
1-octen-3-ol	31.795	1520	MS, RI Std	Fishy, grassy[A] ,sweet earthy[C]
2-ethyl-1-hexanol	33.142	1541	MS, RI	Green rose[A]
4-hepten-1-ol	33.596	1549	MS, RI Std	Fishy [C]
Esters				
ethyl acetate	7.623	692	MS, RI	Fruity orange[A,D] acetic, ethereal odour[C] Caramel, Sweet, Solvent-like, Acid, Buttery, Pungent, Orange[D]
Ketones				
4-methyl-2-heptanone	22.534	1187	MS, RI	ND
1-octen-3-one	26.532	1301	MS, RI Std	Mushroom like [B,C], Metallic, Dirty, Dust, Herb[D]
6-methyl-5-hepten-2-one	27.927	1341	MS, RI Std	Sweet, fruity[A,C,D], fatty[C], Mushroom, Earthy, Vinyl, Rubber, Woody, Blackcurrant, Boiled fruity[D]
Acids				
acetic acid	32.154	1525	MS, RI	pungent odour[C,D], Sour, Vinegar[D]
4-hydroxy Butanoic acid	37.994	1642	MS, RI	ND
2-ethyl Hexanoic acid	46.424	1900	MS, RI	ND
Aromatic compounds				
methylene chloride	9.131	927	MS, RI	Chloroform-like odour[D]
benzaldehyde	33.014	1539	MS, RI	Bitter almond[A,C,D] ,Burnt sugar, Woody[D]
phenol	34.589	1565	MS, RI	Herbal, anisic[A] sweet, tarry odour[C], Medicinal odour[D]

401 [A] Giri et al., 2010; [B] Ganeko et al., 2008; [C] fao.org; [D] pherobase.org

402

403 Although *Laminaria* had the lowest fat content, it contained the highest amount of
404 aldehydes. These volatile compounds can contribute desirable aroma as well as an
405 undesirable rancid odour and flavour during spoilage of fat and fatty foods, due to their
406 low threshold values (Giri, et al., 2010). Straight and branched-chain aldehydes
407 generally provide herbaceous, grassy and pungent aromas, while unsaturated aldehydes
408 are linked with vegetable and fishy notes (Giri et al., 2010)). The formation of
409 aldehydes, including hexanal, heptanal, octanal and nonanal can also be attributed to the
410 decomposition of lipid hydroperoxides and peroxyl radicals. From all this, it could be
411 suggested that, the aldehydes found in this study such as hexanal, heptanal nonanal and
412 2,4-heptadienal may play a major role in determining the volatiles of the seaweed
413 samples.
414 Moreover, branched-chain alcohols like 1-octen-3-ol may contribute significantly to the
415 aroma as they are known to have low odour threshold values. They can be mostly
416 produced by secondary decomposition of hydroxyperoxides of fatty acids, but some of
417 them might also come from carbohydrates by the glycolysis and/or from amino acids
418 via the Ehrlich pathway (Giri et al., 2010). As expected, there were significant
419 differences in the volatile composition between samples, where their overall aroma was
420 enhanced by the presence of aldehydes and alcohols. These compounds have also been
421 found in the volatiles profile of cooked fish or meals containing seafood (Ganeko et al.,
422 2008; Giri et al., 2010).
423
424 ***3.6. Sensory evaluation***
425 Figure 2 shows the spider diagram obtained for the different attributes studied for the
426 aqueous seaweed extracts. Seaweed-like aroma, seafood-like taste and salmon-like taste,
427 where in general, the attributes with the higher scores which could be expected as those

were the attributes more related to "seafood-like". The Friedman test illustrated that panellists were only able to notice significant differences between samples in 3 out of the 9 attributes evaluated. In fact, only *Laminaria sp.* extract was significantly different from all the others in terms of aroma, being the one with the strongest seaweed-like aroma, and the mildest honey-like aroma; and showing the strongest seafood-like taste.

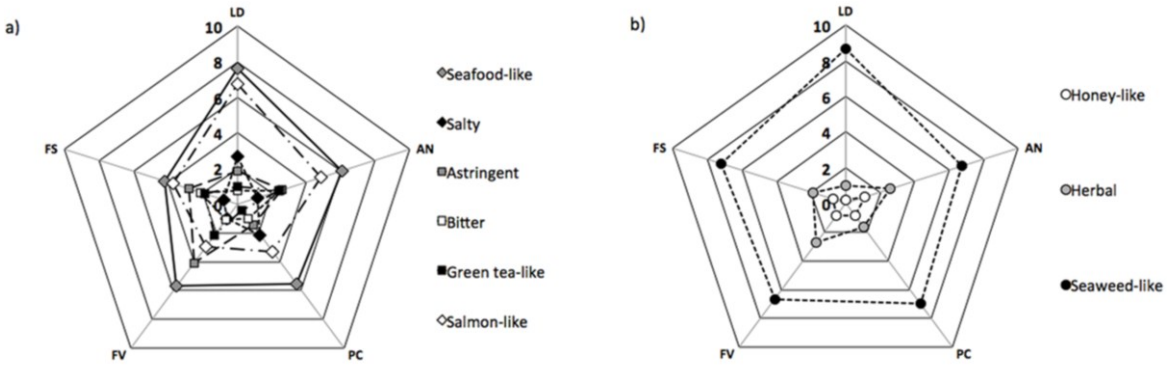


Figure 2. Spider diagram obtained for the different attributes of the different seaweed aqueous extracts. (LD: *Laminaria digitata,* AN: *Ascophyllum nodosum,* PC: *Pelvetia canaliculata,* FV: *Fucus vesiculosus, and* FS: *Fucus spiralis*).

Despite the fact that *Laminaria* showed the highest score for saltiness, as could be expected due to its high concentration in NaCl compared to the other seaweeds, the difference was not significant. The results suggest that the panellists did not associate umami taste with seafood taste or seaweed aroma, as *Laminaria* had the lowest EUC (Table 2). This could be due to the assessors used were untrained subjects unfamiliar with the characteristics of the typical umami taste, however, this type of panel has previously been used for that kind of assessment and though the performance of the untrained panels would not be as good as if they had been trained, they were able to distinguish between samples, (Claperton and Piggott, 1979; Husson & Pagés, 2003)). Therefore its sensory attributes could be mainly due to its high salt content together with high levels of the volatile compounds, hexanal, heptanal, nonanal and 2,4-heptadienal.

3.7. Statistics

Figure 3 illustrates the PCA conducted to simplify the interpretation of the relationships between the seaweed samples and their chemical, volatile and sensory profile. The first three components explain 94 % of the total variance. First principal component (PC1, 54 %) separated *Laminaria* from the other samples, which presented the lower antioxidant activity, highest levels of aldehydes and highest scores for seaweed-like odour and seafood-like taste. The second principal component (PC2, 23 %) differentiated *F. vesiculosus* from the other samples. *F. vesiculosus* possessed the highest nucleotide values as well as the highest concentration of 1-octen-3-ol. Finally, the third principal component (PC3, 17%) differentiated *F. spiralis*. from the other seaweed samples mostly in terms of the amino acid content. As suggested above, the differences in concentrations of the various compounds, such as the high contents of aldehydes and salt in *L. digitata*, or the high content of alcohols (1-octen-3-ol) and nucleotides of *F. vesiculosus*., would be responsible for the different sensory profiles obtained by the panellists.

4. Conclusions.

The chemical composition of the five brown edible seaweeds object of this study was in general terms comparable, with the composition of other brown seaweeds harvested in other areas such as the coast of Spain, Chile, or Norway among others.

The sensory differences observed between the five samples investigated must be attributed to their different chemical compositions. *L. digitataa* and *F. vesiculosus*differ significantly from each other and the other species both in terms of their volatiles and sensory profiles, as well as their chemical composition.

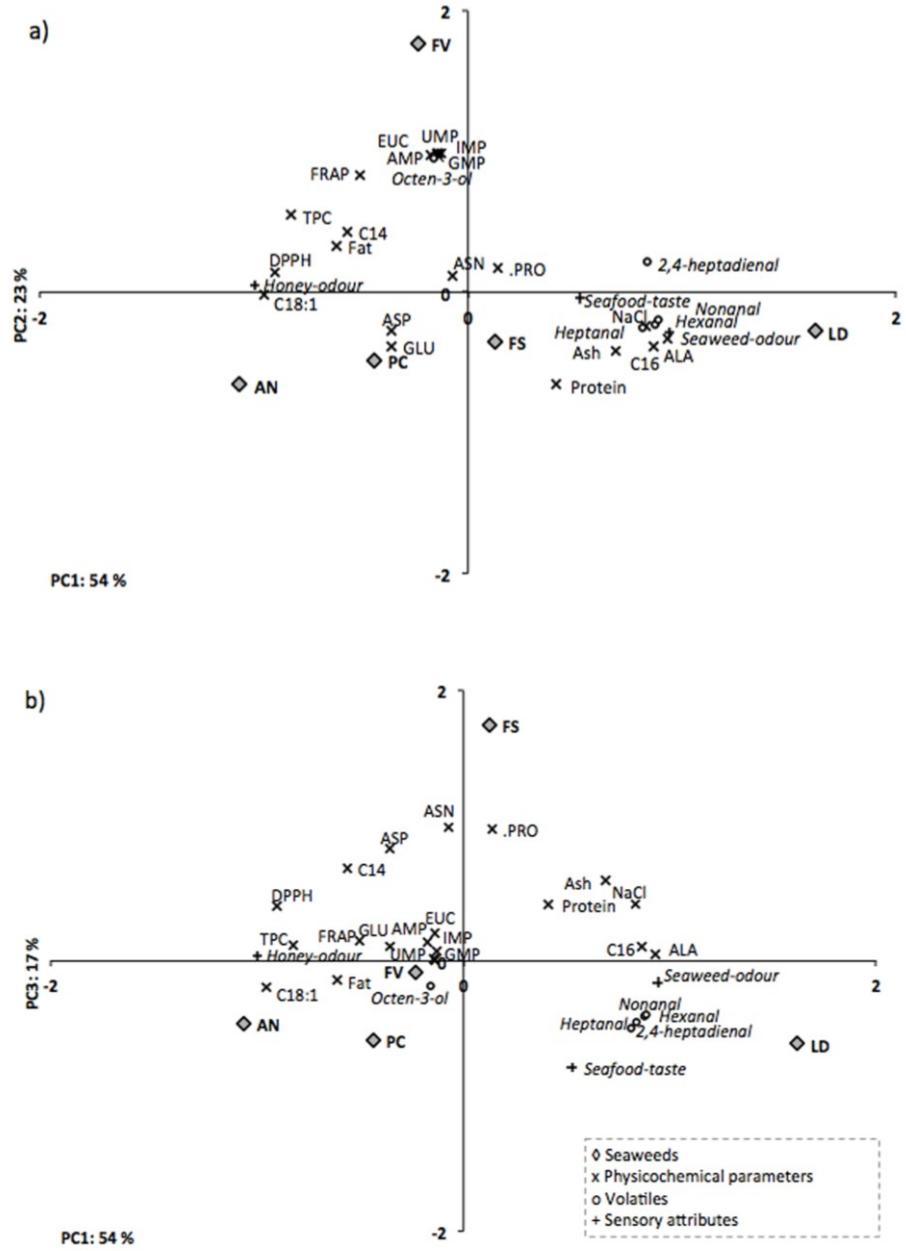


Figure 3. Biplots for the different seaweeds (LD: *Laminaria digitata,* AN: *Ascophyllum nodosum,* PC: *Pelvetia canaliculata,* FV: *Fucus vesiculosus, and* FS: *Fucus spiralis*), depending on their composition: chemical values (ash, NaCl, protein and fat content; antioxidant activity (DPPH, FRAP and TFC), fatty acids and amino acid composition as well as volatiles and sensory attributes. (PC1: 54%, PC2: 23% and PC3: 17%) obtained by means of the PCA analysis.

F. vesiculosus presented high lipid content as well as high level of nucleotides, while *Laminaria* had the lowest lipid and highest salt contents. The fatty acids profile of the

484 samples was dominated by oleic acid, followed by myristic and palmitic acids, although
485 the amounts of them varied between the different seaweeds. The high concentration of
486 nucleotides together with the high amounts of aspartic and glutamic acids may influence
487 the characteristic flavour and taste of *F. vesiculosus*.
488 The high antioxidant activity of the seaweed extracts indicated they could potentially be
489 used as flavour stabilisers specially *Fucus sp*. and *A. nodosum*.
490 Volatiles analysis emphasised the differences between *L. digitata* and *F. vesiculosus*
491 compared to the other species. Besides having the lowest lipid content, *L. digitata*
492 happened to be the seaweed with the highest concentration of lipid-derived aldehydes,
493 and that might be the reason why it resented intense honey-like and seaweed-like odour,
494 as well as an intense seafood-like taste.
495 The importance of these results is the possibility of using locally harvested brown
496 seaweeds, especially *L. digitata* and *F. vesiculosus* which due to their sensory, volatile
497 and chemical composition, could be used to enhance the characteristic umami taste of
498 some foods and/or reduce the need for added salt, as well as providing omponents
499 possessing antioxidant activity.
500

501 **Acknowledgments**
502 Authors would like to thank Frutarom (UK) Ltd and the Technology Strategy Board
503 (TSB), UK for the financial support given to this investigation
504

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