Fernández Segovia, I.; Lerma-García, MJ.; Fuentes López, A.; Barat Baviera, JM. (2018). Characterization of Spanish powdered seaweeds: Composition, antioxidant capacity and technological properties. Food Research International. 111:212-219. https://doi.org/10.1016/j.foodres.2018.05.037

Characterization of Spanish powdered seaweeds: composition, antioxidant capacity and technological properties

Isabel Fernández-Segovia*, María Jesús Lerma-García, Ana Fuentes, Jose M. Barat

Departamento de Tecnología de Alimentos. Universitat Politècnica de València, Camino de Vera s/n, 46022 Valencia, Spain

*Corresponding author.
E-mail address: isferse1@tal.upv.es (I. Fernández-Segovia).

ABSTRACT

This work aimed to characterise four seaweed species: nori (*Phorphyra*), kombu (*Laminaria*), wakame (*Undaria*) and sea spaghetti (*Himanthalia elongata*). Their nutritional composition, total phenolic compounds (TPC), antioxidant capacity, oil and water holding capacity (OHC and WHC), and swelling capacity (SC) were determined. Wakame and nori exhibited the highest proteins contents, rich in essential amino acids and in those related to umami flavour. All the samples had a low lipid content and high ash content values. High fibre levels were observed, especially in kombu. The TPC content and antioxidant capacity of sea spaghetti was significantly higher than in the other samples. The OHC, WHC and SC of the seaweeds demonstrated their potential influence on texture of food products. The incorporation of these seaweeds into different foodstuffs could entail an improvement of the nutritional quality and texture properties, and could also reduce the use of Na and synthetic additives.

Keywords: nutritional composition; total phenolic compounds; antioxidant capacity; physico-chemical properties; seaweeds.

1. Introduction

Seaweeds have been widely consumed in oriental cultures since ancient times. Japan, China or Korea have used seaweeds to prepare many food types. Coastal dwellers in countries such as Indonesia or Malaysia have also consumed seaweeds as an easily available resource. On the contrary in western countries, consumption of this sea vegetable has never been typical. However in recent decades, a growing interest in incorporating seaweeds into diet by non-traditional consumers of this product has been observed (FAO, 2003). This fact has been favoured by the current context of globalization, migration and multiculturalism, together with the current consumer trends, since people seek natural products with a high nutritional value (Astorga-España, Rodríguez-Galdón, Rodríguez-Rodríguez, & Díaz-Romero, 2016; Rupérez, 2002). In Spain, the introduction of seaweeds for culinary use has been limited, but in the last few years new food products with seaweeds have been developed and can be found in supermarkets. In addition, fresh seaweeds, either dehydrated or powdered, among other presentations can be found on the market.

Nori (*Phorphyra*), kombu (*Laminaria*) and wakame (*Undaria*) seaweeds are widely consumed, and the consumption of sea spaghetti (*Himanthalia elongata*) is also important in Europe. Some studies have indicated that including seaweeds in diets has potential benefits for both health status (digestive health, weight management) and chronic diseases, such as cancer or diabetes, among others (Brown et al., 2014; Cao, Wang, Wang, & Ximing, 2016).

Dry seaweeds are rich in proteins, minerals, fibre, phenolic compounds, and are low in fat with polyunsaturated fatty acids; however, the chemical composition of algae varies depending on species, geographic situation, season of harvest or environmental

conditions (Gómez-Ordóñez, Jiménez-Escrig, & Rupérez, 2010; Silva-Marinho, Angelidaki, & Holdt, 2016). In addition, the manufacturing process could influence the chemical composition of marine algae (Mišurcová, Buňka, Vávra-Ambrožová, Machů, Samek, & Kráčmara, 2014). The aim of this work was to characterise seaweeds nori, kombu, wakame and sea spaghetti by evaluating their composition, total phenolic content, antioxidant capacity and technological properties, to assess if its incorporation in food products would improve the nutritional value or bring technological advantages.

2. Materials and methods

2.1. Raw material

Dehydrated and powdered seaweeds from the Atlantic coastal region of Galicia, Spain, were used as raw material. According to the supplier specifications, the powdered product is obtained by a process called micro explosion, which avoids friction maintaining all of its nutritional properties and aroma. Four seaweed types (nori (*Porphyra spp.*) as red seaweed and wakame (*Undaria pinnatifida*), kombu (*Laminaria ochroleuca*) and sea spaghetti (*Himanthalia elongata*) as brown seaweeds) were provided by the company Porto-Muiños (Cerceda, La Coruña, Spain). All the samples were analysed to determine nutritional composition, total phenolic compounds (TPC), antioxidant capacity, as well as oil holding capacity (OHC), water holding capacity (WHC) and swelling capacity (SC).

2.2. Nutritional composition

Contents of lipids, proteins, ashes, dietary fibre, amino acids, cations (sodium, potassium, calcium and magnesium) and chloride anion were determined. All the analyses were carried out in triplicate (n=3).

2.2.1. Analysis of lipid, protein and ash contents

Lipid, protein and ash contents were assayed by AOAC Official Methods 991.36, 928.08, and 920.153, respectively (AOAC, 1997). Lipid content was determined by Soxhlet extraction using petroleum ether. Proteins of samples were quantified by the Kjeldahl method using a factor of 6.25 to convert total nitrogen into protein (Peinado, Girón, Koutsidis, & Ames, 2014). A furnace was used at 550ºC to determine the ash content of the samples.

2.2.2. Dietary fibre

Total dietary fibre (TDF) was analysed according to gravimetric-enzymatic method 991.43 given by AOAC (1997), with some minor modifications. A fibre assay kit was used (TDF100A-1KT, Sigma-Aldrich Co, Steinhem, Germany), which contains α-amylase heat-stable, amyloglucosidase, celite and protease. TDF was quantified by weighing 1.000 g of sample that was suspended in 40 mL of MES (2(N-morpholino) ethanesulfonic acid)/TRIS (tris(hydroxymethyl)aminomethane) buffer adjusting pH at 8.2 and gently shaking. Afterwards 50 µL of α-amylase heat-stable were added and incubated at 95ºC for 15 min in a water bath, shaking every 3 min. Then incubation with 100 µL of protease solution (40 mg of protease in 800 µL of MES/TRIS buffer) at 60ºC for 30 min with continuous shaking was carried out. A volume of 5 mL of hydrochloric acid 0.561 N was added and pH was adjusted at 4.5 with sodium hydroxide 1 N, followed by the addition of 300 µL of amyloglucosidase and incubation at 60ºC for 30

104 min with continuous shaking. Next 225 mL of 96% ethanol, pre-heated at 60ºC, was
105 added and allowed to stand for 1 h at ambient temperature to enable the soluble fibre to
106 precipitate. The digested mixture was vacuum-filtered in the Fibertec 1023 E equipment
107 (Foss System, Hilleroed, Denmark) and the residue was washed twice with 15 mL of
108 78% ethanol and twice with 15 mL of 96% ethanol, and was dried at 103ºC overnight
109 and weighed (weight of TDF + protein + ash). One residue from the sample was
110 analysed for protein content and another residue from the same sample was analysed for
111 ash content by the above-described methods (Section *2.2.1*). TDF contents were
112 calculated by subtracting protein and ash contents.
113
114 *2.2.3. Analysis of Na, K, Ca and Mg contents*
115 The analysis was carried out by ionic exchange chromatography. The ashes
116 obtained by the method described in Section 2.2.1 were dissolved with nitric acid 2 mM
117 in a 10-mL volumetric flask. Afterwards, a dilution of 1:100 was made. This solution
118 was filtered and analysed using an ion exchange column (Metrosep C2, 250/4.0,
119 Methrom®, Herisau, Switzerland) in PC-controlled Compact IC 761 equipment
120 (Methrom®, Herisau, Switzerland) with the following parts: built-in double piston
121 pump, electrically operated injection valve and a temperature-stabilised high
122 performance conductivity detector. The mobile phase was tartaric acid 4 mM and
123 dipicolinic acid 0.75 mM at a rate of 1.0 mL min^{-1}. Separation was monitored using a
124 conductivity detector and data were processed with the IC Net 2.3 software (Methrom®
125 Ltd., Herisau, Switzerland). The identification and quantification of each cation was
126 performed using standard sodim (Na), potassium (K), calcium (Ca) and magnesium
127 (Mg) solutions (Sigma-Aldrich Co, Steinhem, Germany), as described in Section 2.2.5.
128

129 *2.2.4. Analysis of chloride content*

130 Chloride (Cl) content was determined in accordance with the procedure described
131 by Fuentes, Fernández-Segovia, Serra, and Barat (2010) after sample homogenisation in
132 distilled water in an automatic Sherwood Chloride Analyser, Model 926 (Sherwood
133 Scientific Ltd., Cambridge, UK).

134

135 *2.2.5. Analysis of total amino acids*

136 This analysis was performed by HPLC with a UV detector and pre-column
137 derivatization. For amino acid extraction, acidic hydrolysis was conducted according to
138 the method proposed in Commission Regulation (EC) No. 152/2009 (European
139 Commission, 2009), with some minor modifications. A total of 0.1000 g of powdered
140 seaweed was placed in a screw-capped tube and 1 mL of hydrochloric acid 6 N was
141 added. Tubes were heated in a water bath at 100ºC with the screw cap over the tube
142 without closing to avoid explosion. After 1 h, tubes were closed with caps and put in an
143 oven at 110ºC for 23 h. When the hydrolysis had finished, tube caps were opened and
144 the hydrolyzed samples were cooled in an ice-water bath. Then 1 mL of the
145 hydrochloric acid 0.1 N:ethanol mixture (1:1, v:v) was added and filtered. The
146 supernatant was derivatised according to the method described by Concha-Herrera,
147 Lerma-García, Herrero-Martínez, and Simó-Alfonso (2010). The derivatisation reagent
148 consisted of a solution of ortho-phthalaldehyde (OPA) 1.25 x 10^{-2} M and N-
149 acetylcysteine (NAC) 2.5 x 10^{-2} M buffered with 1 M boric acid at pH 9.5. This reagent
150 was protected from light, stored in a refrigerator and weekly renewed. Aliquots of 25
151 μL of the hydrolysates were mixed with 25 μL of sodium hydroxide 10 N, followed by
152 the addition of 1 mL of derivatization reagent. The solution with the derivatised amino

153 acids was filtered through a 0.45-μm nylon filter membrane (Waters, Mildford, MA,
154 USA).

155 Data were collected, stored and analysed using the EZChrom Elite software
156 (Agilent Technologies, Palo Alto, CA, USA). Separations were achieved on a reverse-
157 phase Kromaphase C18 (4.6 x 150 mm, 5 μm particle size) (Scharlab, Barcelona,
158 Spain). A guard column containing the same C18 packing as above was placed in front
159 of the analytical column to protect it from contamination.

160 The solvent system consisted of two eluents: 5 mM citric acid in water adjusted to
161 pH 6.5 with sodium hydroxide (mobile phase A); acetonitrile (mobile phase B). The
162 mobile phases were always filtered through a 0.45-μm nylon 4700 membrane (Waters,
163 Mildford, MA, USA). The mobile phase flow was 1 mL min^{-1} and the used gradient was
164 adapted from the conditions described by Concha-Herrera et al. (2010) (mobile phase
165 A: 95% at time 0, 70% at 30 min, 50% at 35 min and 95% from min 37 until min 45).
166 The injection volume was 20 μL. Chromatograms were monitored at a wavelength of
167 335 nm. Amino acid identification was carried out by comparing the retention times of
168 unknowns with authentic compounds (alanine (Ala), arginine (Arg), aspartic acid (Asp),
169 glutamic acid (Glu), glycine (Gly), histidine (His), isoleucine (Ile), leucine (Leu), lysine
170 (Lys), phenylalanine (Phe), serine (Ser), threonine (Thr), tyrosine (Tyr) and valine (Val)
171 from Sigma-Aldrich Co, Steinhem, Germany) run under the same conditions and by
172 standard addition or "spiking" with standard solutions of 25 mg/L. Amino acid standard
173 solutions were prepared and derivatised following the same procedure described for the
174 samples. Quantification was undertaken using calibration curves of the peak area *vs.*
175 concentration.

176

177 *2.3. Total phenolic compounds and antioxidant capacity*

178

179 *2.3.1. Extract preparation*

180 The method described by Pérez-Jiménez et al. (2008) was followed. From each
181 dehydrated powdered seaweed, 0.5 g was taken and 20 mL of methanol:water (50:50,
182 v/v), acidified at pH 2 with hydrochloric acid, were added and stirred for 1 h at room
183 temperature. Samples were centrifuged (Eppendorf centrifuge 5804 R, Hamburg,
184 Germany) at 5,366 *g* for 10 min and then filtered through a 1.2-µm cellulose filter
185 (Scharlab, Barcelona, Spain). The supernatant was recovered and the residue was
186 extracted with 20 mL of acetone:water (70:30, v/v) by repeating the stirring,
187 centrifugation and filtration steps. The second supernatant was mixed with the first one
188 and this extract was named the methanolic extract. This extract was used to determine
189 total phenolic compounds and antioxidant capacity by the FRAP (Ferric Reducing
190 Antioxidant Power) and ABTS (2,2′-azinobis(3-ethylbenzothiazolin-6-sulphonate)
191 methods.

192

193 *2.3.2. Total phenolic compounds*

194 The total phenolic compounds were determined in the methanolic extracts
195 following the method described by Farvin and Jacobsen (2013). A 100-µL aliquot of the
196 extract was placed inside a test tube to be mixed with 750 µL of 10% (v/v) Folin-
197 Ciocalteu reagent in DW (distilled water) and allowed to stand for 5 min in the darkness
198 at room temperature. Afterwards 750 µL of 5.9% (w/v) sodium bicarbonate in DW were
199 added and allowed to stand for 90 min in the darkness at room temperature. Absorbance
200 was measured at λ = 750 nm. A calibration curve was prepared using gallic acid and the
201 results were expressed as mg gallic acid equivalent/g of powdered seaweed.

202

203 *2.3.3. Antioxidant capacity*

204 Antioxidant capacity is due to different types of compounds which differ in
205 mechanisms of action and can interact synergistically. For this reason, the use of more
206 than one method to determine antioxidant capacity is desirable. In this study the FRAP
207 and the ABTS methods were used to perform *in vitro* assays.

208

209 *2.3.3.1. FRAP method*

210 The method described by Pulido, Bravo, and Saura-Calixto (2000) was followed. A
211 50-μL aliquot of the methanolic extract was mixed with 50 μL of acetate buffer and 900
212 μL of FRAP solution freshly prepared and warmed at 37ºC. Absorbance was measured
213 at λ = 595 nm after 30 min maintaining solutions at 37ºC. A calibration curve was
214 prepared using trolox and the results were expressed as μmol trolox/g of powdered
215 seaweed.

216

217 *2.3.3.2. ABTS method*

218 A decolourisation protocol was used based on different studies (Re, Pellegrini,
219 Proteggente, Pannala, Yang, & Rice-Evans, 1999; Sánchez-Alonso, Jiménez-Escrig,
220 Saura-Calixto, & Borderías., 2008). A weight of 66 mg of ABTS reagent was taken and
221 10 mL of a 2.45 mM potassium persulphate solution were added. This mixture was
222 allowed to stand in the darkness for 24 h at room temperature. Afterwards absorbance
223 was measured at λ = 658 nm and the solution was diluted until an absorbance of
224 0.700±0.020 was reached. A 40-μL aliquot of the methanolic extract was mixed with
225 1.5 mL of the ABTS adjusted solution and absorbance was measured for 10 min at 2-
226 min intervals. A calibration curve was prepared using trolox and the results were
227 expressed as μmol trolox/g of powdered seaweed.

228

229 *2.4. Technological properties*

230

231 The oil holding capacity, water holding capacity and swelling capacity of the four
232 seaweed types were measured according to the methods described by Gómez-Ordóñez
233 et al. (2010) with some minor modifications.

234

235 *2.4.1. Oil holding capacity*

236 A weight of 500 mg of powdered seaweed was placed inside centrifuge tubes. Then
237 30 mL of virgin olive oil were added and gently stirred to completely mix the sample
238 and oil. The mixture was left at room temperature for 24 h in the darkness. After this
239 time, samples were centrifuged at 10,000 rpm for 20 min at 20ºC. The residue was
240 weighed, and the OHC was calculated and expressed as g of oil held/g powdered
241 seaweed.

242

243 *2.4.2. Water holding capacity*

244 The protocol was the same as that described for OHC, except that distilled water
245 was used instead of oil. The results were expressed as g of water held/g powdered
246 seaweed.

247

248 *2.4.3. Swelling capacity*

249 A weight of 500 mg of powdered seaweed was placed inside conical graduated
250 tubes and 10 mL of distilled water were added. After stirring, tubes were covered with
251 aluminium foil and left in the darkness for 18 h at room temperature. Afterwards the

252 volume occupied by the residue was read on the tube scale. The SC was expressed as
253 mL/g powdered seaweed.
254
255 *2.5. Statistical analyses*
256
257 A one-way ANOVA was conducted with the data of the parameters analysed to test
258 if there were any significant differences between the different seaweed types. The least
259 significant difference procedure was used to test for the differences between averages at
260 the 5% significance level. Data are reported as mean±standard deviation. Statistical data
261 processing was performed with the Statgraphics Centurion software (Statpoint
262 Technologies, Inc., Warrenton, VA, USA).
263 A principal component analysis (PCA) was performed with all the evaluated
264 parameters, which meant that the statistical study used 28 variables. All the variables
265 were mean-centred and scaled to unit variance prior to the analysis. For each PCA
266 component, a score for each sample was calculated as a linear combination of the initial
267 data for the variables. The contribution for each parameter to the PCA score was
268 deduced from the loadings for that factor. PCA was performed by version 9.7 of the
269 Unscrambler chemometric software (Camo 2007).
270
271 **3. Results and discussion**
272
273 *3.1. Nutritional composition*
274
275 *3.1.1. Lipid, protein, ash and fibre contents*

Sea spaghetti presented the lowest protein contents (Fig. 1.a) with values of 6.8 g/100 g (dw), followed by kombu, while wakame and nori exhibited significantly higher values with contents above 20 g/100 g (dw). These results agree with other studies in which red seaweeds, such as nori, have been associated with high levels of proteins, and brown seaweeds have shown contents below 15 g/100 g, except for wakame, which usually has higher levels (Fleurence, 1999; Kumar, Sahoo, & Levine, 2015; Kunio & Takahisa, 2000; Peinado et al., 2014; Sánchez-Machado, López-Cervantes, López-Hernández, & Paseiro-Losada, 2004). However, other studies have reported higher protein contents in sea spaghetti and kombu, with values of 14.08 and 28.7 g/100 g, respectively (Gómez-Ordóñez et al., 2010). These differences could be due to the variability in the seaweed composition throughout the year. These results confirm that the studied seaweeds, especially nori and wakame, are a good source of proteins, and are comparable to some terrestrial vegetables with high protein contents. It is important to note that the Kjeldahl method measures the nitrogen content. Therefore, the protein content may have been over-estimated due to measurement of free amino acids and other non-protein N-compounds which are also quantified by Kjeldahl.

Lipid content varied between 0.08 g/100 g (nori) and 1.1 g/100 g (wakame) (Fig. 1.a), which demonstrates the low caloric value of seaweeds. It is important to note that, although lipid content was low, they are rich in polyunsaturated fatty acids, as demonstrated in other studies (Peinado et al., 2014; Sánchez-Machado et al., 2004). This low lipid content, together with the excellent fatty acid profile, is an advantage of using marine algae as an ingredient in food products. A study carried out on dry pasta made with semolina and seaweed blends, showed that addition of wakame increased the levels of ω-3 fatty acids, improving the nutritional quality of the final product (Prabhasankar et al., 2009).

301 Regarding ash content, the four samples exhibited significantly different values,
302 which ranged between 24 and 34 g/100 g (Fig. 1.a). Nori showed the lowest ash content,
303 which agrees with other studies which have evidenced that red seaweeds contain lower
304 values than brown ones (Kumar et al., 2015; Rupérez, 2002). Ash is the second major
305 fraction after dietary fibre, which is due to seaweeds being rich in minerals, such as
306 sodium, potassium, calcium, magnesium (described below), phosphorous, iron and also
307 contain iodine, chloride and sulphate ions (Gómez-Ordóñez et al., 2010; Cofrades,
308 López-López, Solas, Bravo, & Jiménez-Colmenero, 2008).
309 Fig. 1.a shows the fibre content of the four analysed samples. It is important to note
310 the high fibre content showed by all the samples. Kombu exhibited the highest value
311 (over 40% dry weight). The recommended daily intake of fibre is about 25-30 g/day
312 (FAO, 2017). However, a study carried out in Spain showed that the average fibre
313 intake in the Spanish population was of 16.24 g/day in the period 2004-2008 (Ruiz-
314 Roso & Pérez-Olleros, 2010). A recent work also showed that the intake of fibre in
315 Spanish adults (12.59 g/day) is below the recommended values (González-Rodríguez et
316 al., 2017). These data confirm the importance of incorporating seaweeds into food with
317 low or null fibre content, like meat and fish products, as it represents an important
318 contribution of such compounds.
319
320 *3.1.2. Sodium, potassium, calcium, magnesium and chloride contents*
321 Fig. 1.b shows the contents of the four cations and chloride anion. Sodium contents
322 were high, and wakame obtained a significantly higher value than the other seaweeds.
323 However, the K content of this marine plant was very low, while the rest of the samples,
324 especially kombu and sea spaghetti showed high K contents. From a nutritional point of
325 view it is important for the Na/K ratio to be low since diets rich in Na or with a high

326 Na/K ratio have been associated with hypertension problems and other diseases
327 (Rupérez, 2002). The Na/K ratios were 1.3, 0.2, 9.5 and 0.42 for nori, kombu, wakame
328 and sea spaghetti, respectively. All the seaweeds except for wakame showed an
329 excellent Na/K ratio. The low K content found in wakame agrees with the results
330 reported by Flores, Dobbs, and Dunn (2015), although values above 8 g K/100 g have
331 been found in other studies (Rupérez, 2002).

332 In Spain, consumption of Na is normally higher than that recommended by the
333 World Health Organization (5 g of salt). For this reason, reducing salt consumption
334 from 9.8 to 5 g is a priority objective of the NAOS Strategy (Strategy for Nutrition,
335 Physical Activity and Obesity Prevention) presented by the Spanish Agency of
336 Consumption, Food Safety and Nutrition (AECOSAN). To achieve this goal, among
337 other actions, the Ministry of Health has asked industries to reduce the salt content of
338 processed products as they are the main source of Na in diet. Some strategies studied to
339 reduce Na in processed foods include the partial replacement of Na with K or using
340 flavour enhancers. The incorporation of seaweeds into processed products can be
341 positive since they have a high K content together with some amino acids and other
342 compounds that can act as flavour enhancers, which would reduce the amount of added
343 salt.

344 No significant differences were observed in the Ca contents among the different
345 samples. Seaweeds are also an important source of Ca. Wakame showed a significantly
346 higher Mg content compared to the other three samples. These results agree with other
347 studies, where the Mg content of wakame was in some cases almost double that in
348 kombu or nori (Flores et al., 2015; Rupérez, 2002).

349 The lowest Cl content was found in the nori sample, which agrees with some
350 studies that have stated that red seaweeds have a lower content of this anion compared

351 to brown ones since red ones live in deeper waters with a lower salinity level. Sea
352 spaghetti had the highest Cl content and the highest percentage in ashes (33.85%),
353 which agrees with other studies in which brown seaweeds have reached chloride levels
354 in ashes of up to 33-37% (Gómez-Ordóñez et al., 2010).

355 From these results it can be concluded that the seaweeds evaluated in this study are
356 a good source of minerals, even better than some terrestrial vegetables, and could be an
357 interesting alternative to reduce Na in processed food.

358

359 *3.1.3. Total amino acid content*

360 The chromatograms obtained for the different seaweeds are observed in Fig. 2.
361 Fourteen amino acids were detected and quantified. Amino acids Phe and Leu appeared
362 at the same retention time, peaks overlapped and they could not be quantified
363 separately. For this reason, the content of these amino acids was expressed as the sum of
364 both compounds. The contents of the total amino acids quantified in the samples are
365 shown in Fig. 3. The amino acid contents in nori and wakame were significantly higher
366 than in the other seaweeds (Fig. 3.a). Sea spaghetti had the lowest level of amino acids,
367 followed by kombu, which was due to the difference in the above-mentioned protein
368 contents, where nori and wakame had higher protein levels compared to kombu and sea
369 spaghetti.

370 In Fig. 3.b the amino acid contents refer to protein weight, and the differences in
371 the amino acid composition of the proteins in seaweeds can be observed. The proteins
372 of all seaweeds were rich in essential amino acids (His, Thr, Val, Ile, Phe, Leu and Lys),
373 which agrees with other studies (Astorga-España et al., 2016; Mišurcová et al., 2014;
374 Syad, Shunmugiah, & Kasi, 2013). This means that the proteins of the seaweeds can be
375 considered to be of high quality, especially nori and wakame. In a study carried out on

dry pasta, incorporation of wakame into its formulation improved the amino acid profile (Prabhasankar et al., 2009). Regarding non-essential amino acids, Asp and Glu contents in the four samples were high. Both these amino acids have been related to umami flavour, so these play an important role in seaweeds being used as flavour enhancers. In other works high contents of these compounds have been found, with values of up to 26% of total amino acids in some seaweeds (Dawczynski, Schubert, & Jahreis, 2007; Peinado et al., 2014; Syad et al., 2013). It is noteworthy that glutamine and asparagine are converted into Glu and Asp, respectively, during hydrolysis (Fleurence, 1999). For this reason, there is a simultaneous quantification of Glu and Asp with their amides. High Ala contents were also found in all the samples. Mišurcová et al. (2014) stated that Ala, Glu and Gly are considered to be the main components of seaweed flavour.

Although the amino acids found in this research were similar to those found in other works, there were differences in the amino acid profiles, which could be caused by distinct environmental growth conditions, such as geographic area, season, temperature or nutrient content in water, as reported elsewhere (Marsham, Scott, & Tobin, 2007). It can be concluded that the four seaweeds, especially nori and wakame, are a major source of amino acids.

3.2. Total phenolic compounds and antioxidant capacity

3.2.1 Total phenolic compounds.

The obtained results are shown in Table 1. No significant differences were found between nori, kombu and wakame. However, the total phenolic compounds content obtained in sea spaghetti was significantly higher than in the rest of the samples. In other studies carried out on different seaweeds, high TPC values in sea spaghetti have

401 also been found (higher than 30 mg phloroglucinol/g) (Jiménez-Escrig, Gómez-
402 Ordóñez, & Rupérez, 2012; Rajauria, Barry, & Abu-Ghannam, 2016). The proportion of
403 this type of compounds varies vastly depending on the seaweed species, with a wide
404 variability within one same species since phenolic content is strongly affected by
405 environmental factors, such as light, saltiness or temperature. This is because seaweeds
406 synthesise these compounds to protect themselves against stress conditions (Connan,
407 Deslandes, & Ar Gall, 2007; Kumar et al., 2015; O'Sullivan et al., 2011).
408
409 *3.2.2 Antioxidant capacity*
410 Two methods (FRAP and ABTS) were used to determine the *in vitro* antioxidant
411 properties of the different seaweed types The results are shown in Table 1. Sea spaghetti
412 exhibited a significantly higher radical scavenging capacity and reducing power
413 compared with nori, kombu or wakame, by both methodologies. These results agree
414 with a study carried out on meat products with seaweeds, which showed a greater
415 antioxidant capacity for products with sea spaghetti (3.69 μmol trolox/g meat product),
416 while those with nori and wakame had a lower trolox concentration (1.18 and 1.09 μmol
417 trolox/g meat product, respectively), and no significant differences were found between
418 them (López-López et al., 2009). Other studies have shown the antioxidant activity of
419 polyphenols extracted from seaweeds (Rajauria, Jaiswal, Abu-Ghannam, & Gupta,
420 2013; Rodríguez-Bernaldo de Quirós, Frecha, Vidal, & López, 2010). This agrees with
421 the findings of this work since the higher TPC found in sea spaghetti could have
422 entailed the greater antioxidant capacity of this seaweed species. It is important to note
423 that, in addition to polyphenols, seaweeds contains other antioxidant compounds, such
424 as polysaccharides, pigments, proteins or peptides (Agregán, Munekata, Domínguez,
425 Carballo, Franco, & Lorenzo, 2017; Farvin & Jacobsen, 2013). Thus the seaweeds

426 studied herein, especially sea spaghetti, can be considered functional ingredients of
427 much interest for food formulations. Their use could increase shelf-life of food products
428 and could have a positive effect on the human health due to their high antioxidant
429 capacity.
430
431 *3.3. Technological properties*
432
433 The values of oil holding capacity, water holding capacity and swelling capacity of
434 the four seaweed types are shown in Fig. 4. Similar OHC values were observed for all
435 the seaweeds considered herein. However, these values were slightly higher than those
436 found in other studies conducted on different seaweed species (values ranging from 1.22
437 to 1.61 g/g dry weight) (Gómez-Ordóñez et al., 2010).
438 The highest WHC value was obtained for wakame, followed by kombu. In this
439 case, the reported values were also slightly higher (around three units) than those
440 reported by other authors (Gómez-Ordóñez et al., 2010). This parameter, like OHC, is
441 of much importance from a technological point of view as it directly affects texture.
442 Several studies have been carried out using seaweeds, such as wakame, nori or sea
443 spaghetti, in meat products, with improvements observed in oil and water binding
444 properties, which thus improve the texture of products (López-López et al., 2009;
445 Cofrades et al., 2008).
446 SC followed a similar pattern to WHC, for which wakame gave the highest value,
447 followed by kombu, spaghetti and nori.
448 Thus, it can be concluded that these properties imply an added value of seaweeds in
449 the food industry because, in addition to nutritional interest, they could replace some

450 additives like phosphates and other compounds that have been widely used to improve
451 the texture and juiciness of meat and fish products.
452
453 *3.4. Principal Component Analysis*
454
455 The global variability among the different seaweed types studied in this work, was
456 analysed by a Principal Component Analysis (PCA), where all the parameters evaluated
457 were the variables. The results are shown in a loading plot (Fig. 5.a) and a score plot
458 (Fig. 5.b). The first two principal components explained 96% of total variation. The
459 most important principal component (PC1) explained 82% of total variation. Fig. 5.a
460 depicts how all the variables related to antioxidant capacity (TPC, FRAP and ABTS)
461 had positive loadings on PC1 and PC2. The sample whose scores are in that area (Fig.
462 5.b) is sea spaghetti, which demonstrates the remarkable difference in these properties
463 compared with the other seaweed types. In the same way, kombu is characterised
464 mainly for its high fibre content and, to a lesser extent, for its high K contents. The
465 majority of amino acids and protein contents have negative loadings on PC1 and
466 positive ones on PC2, which occurs with wakame and nori in the score plot. This
467 confirms that these components were higher in these seaweeds (especially Thr and
468 Phe+Leu), and could discriminate between these and the other evaluated seaweeds. The
469 other parameters related to composition (ash, lipid, mineral contents) and those related
470 to technological properties are close to 0 on both axes, centred between the different
471 seaweeds. This reveals that these parameters were not important in the discrimination
472 between samples.
473

4. Conclusions

The four seaweeds, especially nori and wakame, have high protein contents rich in essential amino acids and have a high proportion of aspartic and glutamic acid (both related to umami flavour), which is interesting for the use of seaweeds as flavour enhancers. Nori, kombu, wakame and sea spaghetti are rich in minerals (high ash content) and low in calories (low lipid contents). They exhibit high Na, K, Ca, Mg and chloride values. The Na/K ratio is especially low in kombu and sea spaghetti, so their incorporation into processed food is a good alternative to reduce the amount of Na in these products.

The high fibre contents found, especially in kombu, imply that the incorporation of these seaweeds into low-fibre products, such as fish or meat, could be a means of increasing fibre intake and would improve the nutritional value of these products.

The four evaluated seaweeds, especially sea spaghetti, are a source of natural antioxidants, as demonstrated by the high total phenolic compounds contents and the high antioxidant capacity found. Therefore, these seaweeds could substitute the synthetic antioxidants commonly used in food formulation.

All the seaweeds characterised in this study exhibited important oil and water holding properties, especially wakame, which also displayed the highest swelling capacity. These properties make seaweeds suitable to substitute some chemical additives used to improve texture and juiciness, and to reduce exudates in food products.

In general, the incorporation of nori, wakame, kombu and/or sea spaghetti into food products would imply added value thanks to their high protein, mineral and fibre contents and their low energy and sodium content. With the results obtained herein, the

499 best combination of these seaweeds could be defined by producers, depending on the
500 objective pursued.
501
502 **Conflict of interests**
503
504 There is not any conflict of interests.
505
506 **Acknowledgements**
507
508 The reported experiment forms part of a project financially supported by the
509 Universitat Politècnica de València ("SaPesAl" (UPV-FE-2014-55)), which the authors
510 gratefully acknowledge. M.J. Lerma-García thanks the Universitat Politècnica de
511 València for a postdoctoral contract (PAID-10-14).
512
513 **References**
514
515 AOAC (1997). *Official methods of analysis*, (16th ed.). Washington: Association of
516 Official Analytical Chemists.
517 Agregán, R., Munekata, P. E., Domínguez, R., Carballo, J., Franco, D., & Lorenzo, J.
518 M. (2017). Proximate composition, phenolic content and *in vitro* antioxidant activity
519 of aqueous extracts of the seaweeds *Ascophyllum nodosum*, *Bifurcaria bifurcata* and
520 *Fucus vesiculosus*. Effect of addition of the extracts on the oxidative stability of
521 canola oil under accelerated storage conditions. *Food Research International, 99,*
522 986-994.

523 Astorga-España, M. A., Rodríguez-Galdón, B., Rodríguez-Rodríguez, E. M., & Díaz-
524 Romero, C. (2016). Amino acid content in seaweeds from the Magellan Straits
525 (Chile). *Journal of Food Composition and Analysis, 53*, 77–84.
526 Brown, E. M., Allsopp, P. J., Magee, P. J., Gill, C. I. R., Nitecki, S., Strain, C. R., &
527 McSorley, E.M. (2014). Seaweed and human health. *Nutrition Reviews, 72*, 205–
528 216.
529 Cao, J., Wang, J., Wang, S., & Ximing, X. (2016). Porphyra species: A Mini-Review of
530 its pharmacological and nutritional properties. *Journal of Medicinal Food, 19*(2),
531 111–119.
532 Cofrades, S., López-López, I., Solas, M. T., Bravo, L., & Jiménez-Colmenero, F.
533 (2008). Influence of different types and proportions of added edible seaweeds on
534 characteristics of low-salt gel/emulsion meat systems. *Meat Science, 79*, 767-776.
535 Concha-Herrera, V., Lerma-García, M. J., Herrero-Martínez, J. M., & Simó-Alfonso, E.
536 F. (2010). Classification of vegetable oils according to their botanical origin using
537 amino acid profiles established by High Performance Liquid Chromatography with
538 UV-vis detection: A first approach. *Food Chemistry, 120*, 1149-1154.
539 Connan, S., Deslandes, E., & Ar Gall, E. (2007). Influence of day-night and tidal cycles
540 on phenol content and antioxidant capacity in three temperate intertidal brown
541 seaweeds. *Journal of Experimental Marine Biology and Ecology, 349*, 359-369.
542 Dawczynski, C., Schubert, R., & Jahreis, G. (2007). Amino acids, fatty acids, and
543 dietary fibre in edible seaweed products. *Food Chemistry, 103*(3), 891-899.
544 European Commission (2009). Regulation (EC) No 152/2009 of 27 January 2009 laying
545 down the methods of sampling and analysis for the official control of feed.
546 FAO (Food and Agriculture Organization). Necesidades Nutricionales. (2017).
547 http://www.fao.org/docrep/014/am401s/am401s03.pdf Accessed 03.06.17.

548 FAO (Food and Agriculture Organization). A guide to the seaweed industry. (2003).
549 http://www.fao.org/docrep/014/am401s/am401s03.pdf Accessed 30.03.18.
550 Farvin, S. K. H., & Jacobsen, C. (2013). Phenolic compounds and antioxidant activities
551 of selected species of seaweeds from Danish coast. *Food Chemistry, 138*, 1670-
552 1681.
553 Fleurence, J. (1999). Seaweed proteins: biochemical, nutritional aspects and potential
554 uses. *Trends in Food Science & Technology, 10*, 25-28.
555 Flores, S. R. L., Dobbs, J., & Dunn, M.A. (2015). Mineral nutrient content and iron
556 bioavailability in common and Hawaiian seaweeds assessed by an in vitro
557 digestion/Caco-2 cell model. *Journal of Food Composition and Analysis, 43*, 185–
558 193.
559 Fuentes, A., Fernández-Segovia, I., Serra, J. A., & Barat, J. M. (2010). Development of
560 a smoked sea bass product with partial sodium replacement. *LWT - Food Science*
561 *and Technology, 43*(9), 1426–1433.
562 Gómez-Ordóñez, E., Jiménez-Escrig, A., & Rupérez, P. (2010). Dietary fibre and
563 physicochemical properties of several edible seaweeds from the northwestern
564 Spanish coast. *Food Research International, 43,* 2289-2294.
565 González-Rodríguez, L. A., Perea J. M., Aranceta-Bartrina, J., Gil, A., González-Gross,
566 M., Serra-Majem, Ll., Varela-Moreiras, G., & Ortega, R. M. (2017). Intake and
567 dietary food sources of fibre in Spain: differences with regard to the prevalence of
568 excess body weight and abdominal obesity in adults of the ANIBES study.
569 *Nutrients, 9*(326), 1-22.
570 Jiménez-Escrig, A., Gómez-Ordóñez, E., & Rupérez, P. (2012). Brown and red
571 seaweeds as potential sources of antioxidant nutraceuticals. *Journal of Applied*
572 *Phycology, 24*(5), 1123-1132.

573 Kumar, S., Sahoo, D., & Levine, I. (2015). Assessment of nutritional value in a brown
574 seaweed *Sargassum wightii* and their seasonal variations. *Algal Research, 9*, 117-
575 125.
576 Kunio, S., & Takahisa, N. (2000). Identification of an antihypertensive peptide from
577 peptic digest of wakame (*Undaria pinnatifida*). *Journal of Nutritional Biochemistry,*
578 *11*(9), 450-454.
579 López-López, I., Bastida, S., Ruiz-Capillas, C., Bravo, L., Larrea, M. T., Sánchez-
580 Muniz, F., Cofrades, S., & Jiménez-Colmenero, F. (2009). Composition and
581 antioxidant capacity of low-salt meat emulsion model systems containing edible
582 seaweeds. *Meat Science, 83*, 492-498.
583 Marsham, S., Scott, G. W., & Tobin, M. L. (2007). Comparison of nutritive chemistry
584 of a range of temperature seaweeds. *Food Chemistry, 100*, 1331–1336
585 Mišurcová, L., Buňka, F., Vávra-Ambrožová, J., Machů, L., Samek, D., & Kráčmara, S.
586 (2014). Amino acid composition of algal products and its contribution to RDI. *Food*
587 *Chemistry, 151*, 120-125.
588 O'Sullivan, A. M., O'Callaghan, Y. C., O'Grady, M. N., Queguineur, B., Hanniffy, D.,
589 Iroy, D. J., Kerry, J. P., & O'Brien, N. M. (2011). *In vitro* and cellular antioxidant
590 activities of seaweed extracts prepared from five brown seaweeds harvested in
591 spring from the west coast of Ireland. *Food Chemistry, 126*, 1064-1070.
592 Peinado, I., Girón, J., Koutsidis, G., & Ames, J. M. (2014). Chemical composition,
593 antioxidant activity and sensory evaluation of five different species of brown edible
594 seaweeds. *Food Research International, 66*, 36-44.
595 Pérez-Jiménez, J., Arranz, S., Tabernero, M., Díaz-Rubio, M. E., Serrano, J., Goñi, I., &
596 Saura-Calixto, F. (2008). Updated methodology to determine antioxidant capacity in

plant foods, oils and beverages: Extraction, measurament and expression of results. *Food Research International, 41*, 274-285.

Prabhasankar, P., Ganesan, P., Bhaskar, N., Hirose, A., Sthepen, N., Gowda, L. R., Hosokawa, M., & Miyashita, K. (2009). Edible Japanese seaweed, wakame (*Undaria pinnatifida*) as an ingredient in pasta: Chemical, functional and structural evaluation. *Food Chemistry, 115*, 501-508.

Pulido, R., Bravo, L., & Saura-Calixto, F. (2000). Antioxidant activity of dietary polyphenols as determined by a modified ferric reducing/antioxidant power assay. *Journal of Agricultural and Food Chemistry, 48*, 3396-3402.

Rajauria, G., Barry, F., & Abu-Ghannam, N. (2016). Identification and characterization of phenolic antioxidant compounds from brown Irish seaweed *Himanthalia elongata* using LC-DAD-ESI-MS/MS. *Innovative Food Science and Emerging Technologies, 37*, 261-268.

Rajauria, G., Jaiswal, A. K., Abu-Ghannam, N., & Gupta, S. (2013). Antimicrobial, antioxidant and free radical-scavenging capacity of brown seaweed *Himanthalia elongatae* from western coast of Ireland. *Journal of Food Biochemistry, 37*(3): 322-335.

Re, R., Pellegrini, N., Proteggente, A., Pannala, A., Yang, M., & Rice-Evans, C. (1999). Antioxidant activity applying an improved ABTS radical cation decolorization assay. *Free Radical Biology and Medicine, 26*, 1231-1237.

Rodríguez-Bernaldo de Quirós, A., Frecha, F. S., Vidal, P. A., & López, H. J. (2010). Antioxidant compounds in edible brown seaweeds. *European Food Research and Technology, 231*(3), 495-498.

620 Ruiz-Roso, B., & Pérez-Olleros, L. (2010). Avance de resultados sobre consumo de
621 fibra en España y beneficios asociados a la ingesta de fibra insoluble. *Revista*
622 *Española de Nutrición Comunitaria, 16*(3), 147-153.
623 Rupérez, P. (2002). Mineral content of edible marine seaweeds. *Food Chemistry, 79*,
624 23-26.
625 Sánchez-Alonso, I., Jiménez-Escrig, A., Saura-Calixto, F., & Borderías, J. (2008).
626 Antioxidant protection of white grape pomace on restructured fish products during
627 frozen storage. *LWT-Food Science and Technology International, 41*, 42-50.
628 Sánchez-Machado, D. I., López-Cervantes, J., López-Hernández, J., & Paseiro-Losada,
629 P. (2004). Fatty acids, total lipid, protein and ash contents of processed edible
630 seaweeds. *Food Chemistry, 85*, 439-444.
631 Silva-Marinho, G., Angelidaki, I., & Holdt, S. L. (2016). *Bioremediation capacity,*
632 *nutritional value and biorefining of macroalga Saccharina latissima*. Denmark:
633 Kgs. Lyngby: Technical University of Denmark, DTU Environment.
634 Syad, A. N., Shunmugiah, K. P., Kasi, P. D. (2013). Seaweeds as nutritional
635 supplements: Analysis of nutritional profile, physicochemical properties and
636 proximate composition of *G. acerosa* and *S. wightii*. *Biomedicine & Preventive*
637 *Nutrition, 3*, 139-144.
638

639 **Figure captions**

640

641 **Fig. 1. (a)** Protein, lipid, ash and fibre contents and **(b)** Na, K, Ca, Mg and chloride
642 contents of powdered seaweeds, expressed as g/100 g (dry weight) (means and standard
643 deviations, *n=3*). Bars indicate standard deviation.

644

645 **Fig. 2.** Chromatograms obtained in the amino acids analysis by HPLC for the four
646 sample types. Codes: Alanine (Ala), arginine (Arg), aspartic acid (Asp), glutamic acid
647 (Glu), glycine (Gly), histidine (His), isoleucine (Ile), leucine (Leu), lysine (Lys),
648 phenylalanine (Phe), serine (Ser), threonine (Thr), tyrosine (Tyr) and valine (Val)).

649

650 **Fig. 3.** Total amino acids content of powdered seaweeds expressed as **(a)** mg/100 g
651 seaweed (dry weight) and **(b)** mg/100 g protein (means and standard deviations, *n=3*).
652 Bars indicate standard deviation. Codes as in Fig. 2.

653

654 **Fig. 4.** Values of oil holding capacity (OHC) and water holding capacity (WHC),
655 expressed as g of oil or water/g (dry weight), and swelling capacity (SC) expressed as
656 mL/g (dry weight) of powdered seaweeds (means and standard deviations, *n=3*). Bars
657 indicate standard deviation.

658

659 **Fig. 5.** Principal components analysis (PCA) for nori, kombu, wakame and sea
660 spaghetti. **(a)** Loading plot **(b)** Score plot. (TPC: Total phenolic compounds; FRAP:
661 Ferric Reducing Antioxidant Power; ABTS: 2,2′-azinobis(3-ethylbenzothiazolin-6-
662 sulphonate; OHC and WHC: oil and water holding capacity, respectively; SW: Swelling
663 capacity. Rest of the codes given in Fig. 2).

664 **Table 1**

665 Total phenolic compounds (TPC) (expressed as mg gallic acid equivalent (GAE)/g dry
666 weight of powdered seaweeds) and antioxidant capacity determined by the FRAP and
667 ABTS methods (expressed as μmol trolox/g dry weight of powdered seaweeds) (means
668 and standard deviations, *n=3*).

	TPC	Antioxidant capacity (μmol trolox/g)	
	(mg GAE/g)	**FRAP**	**ABTS**
Nori	2.91±0.07[a]	2.1±0.2[a]	7.2±0.8[a]
Kombu	1.7±0.8[a]	3.8±0.8[a]	12.3±0.2[a]
Wakame	2.6±0.4[a]	4.91±0.02[a]	5.3±0.3[a]
Sea Spaghetti	18±2[b]	41.1±0.8[b]	65±6[b]
	***	***	***

669 Different letters in the same column indicate significant differences.
670 *** $p < 0.001$
671
672

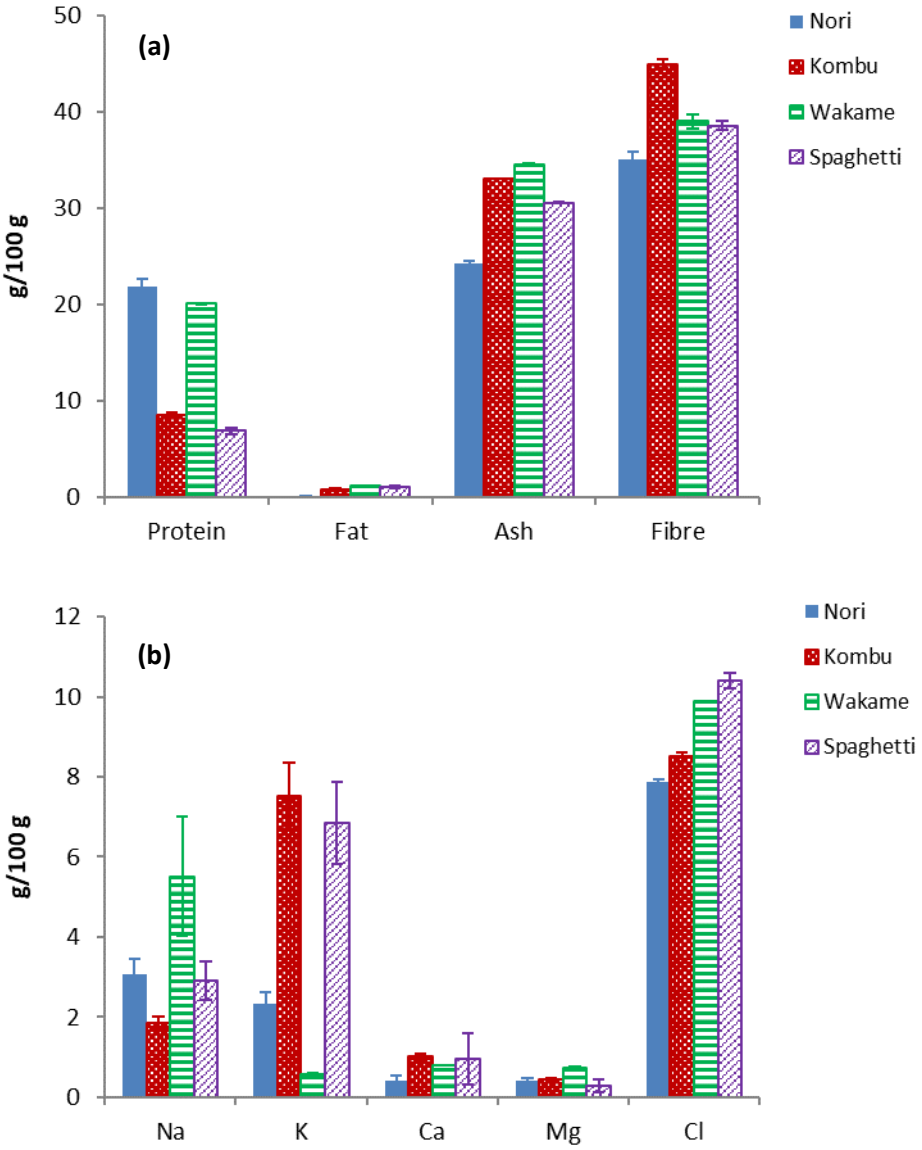

(a)
g/100 g
50
40
30
20
10
0
Protein
Fat
Ash
Fibre
Nori
Kombu
Wakame
Spaghetti
(b)
g/100 g
12
10
8
6
4
2
0
Na
K
Ca
Mg
Cl
Nori
Kombu
Wakame
Spaghetti

Fig. 1.

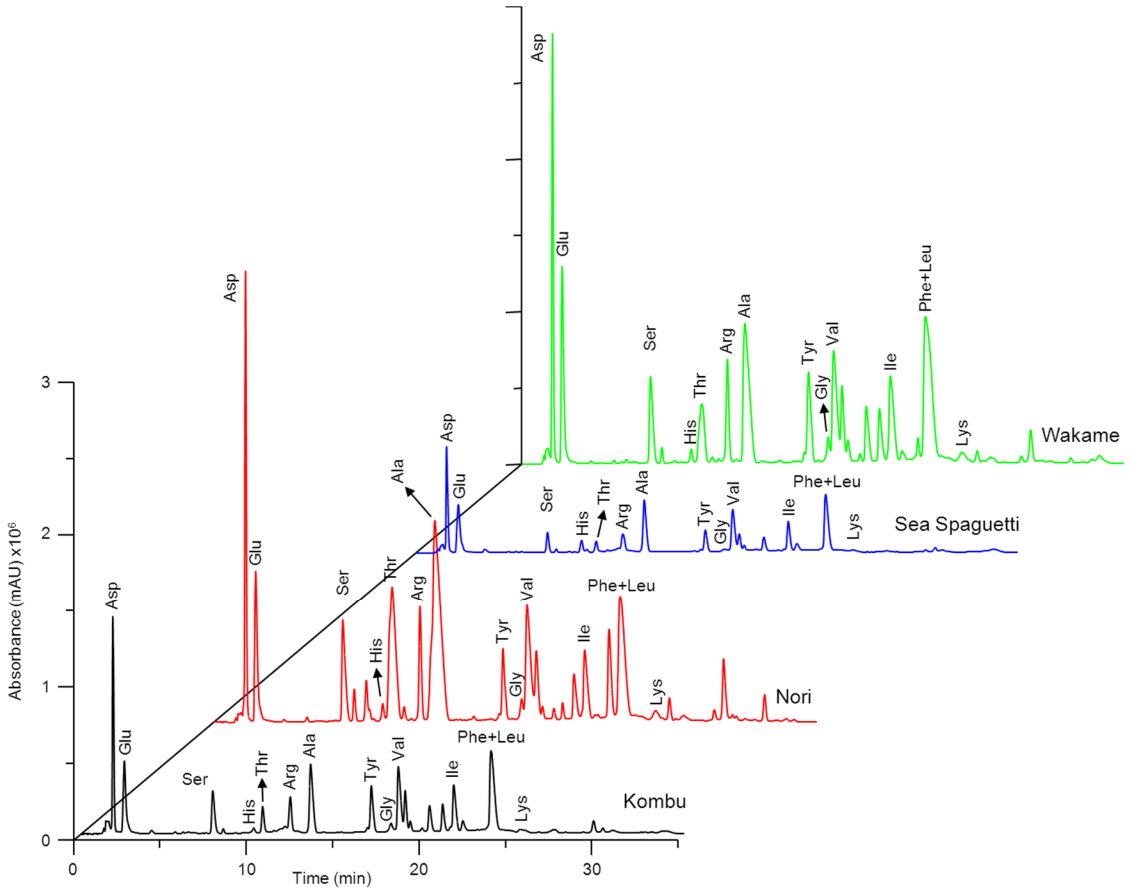
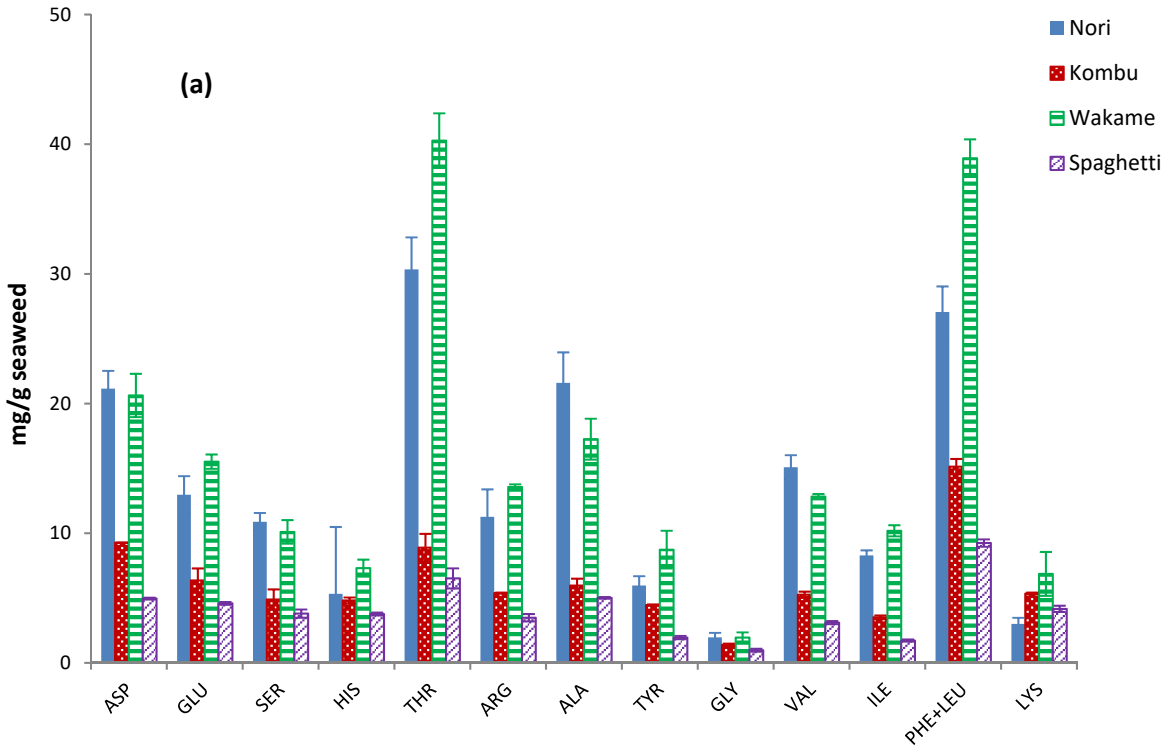
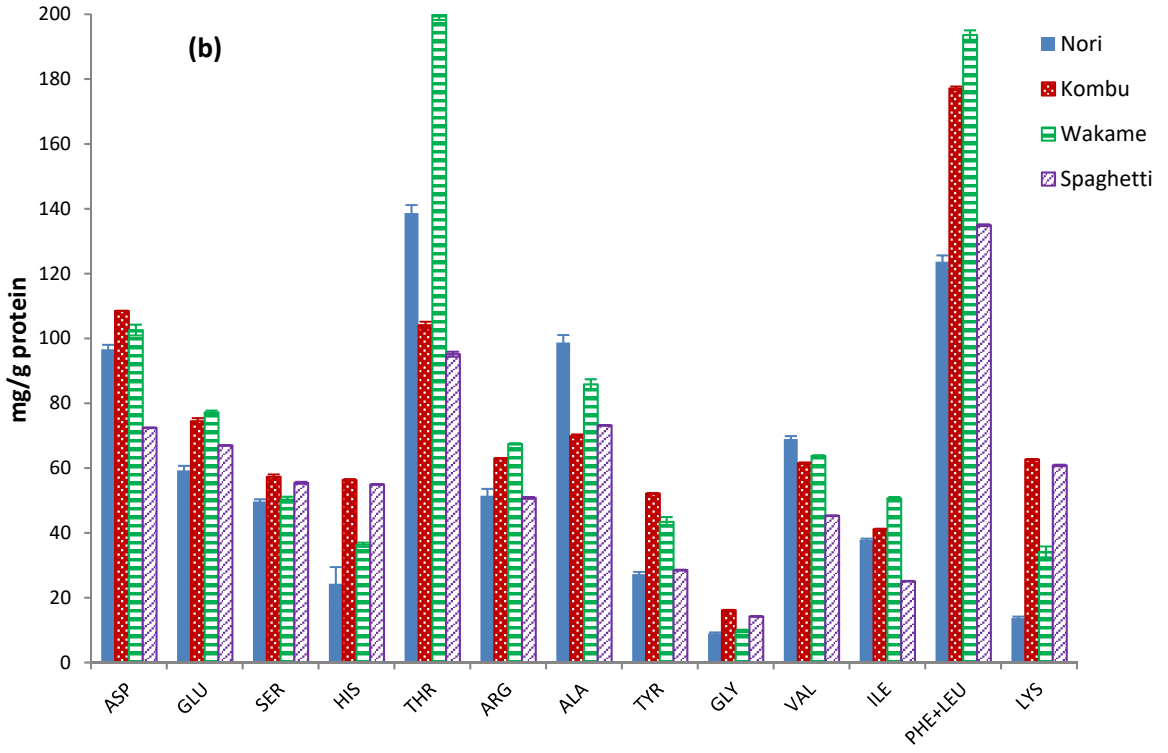

Absorbance (mAU) x10⁶
Time (min)
Wakame
Sea Spaguetti
Nori
Kombu
Asp
Glu
Ser
His
Thr
Arg
Ala
Tyr
Gly
Val
Ile
Phe+Leu
Lys

Fig. 2.

699



700



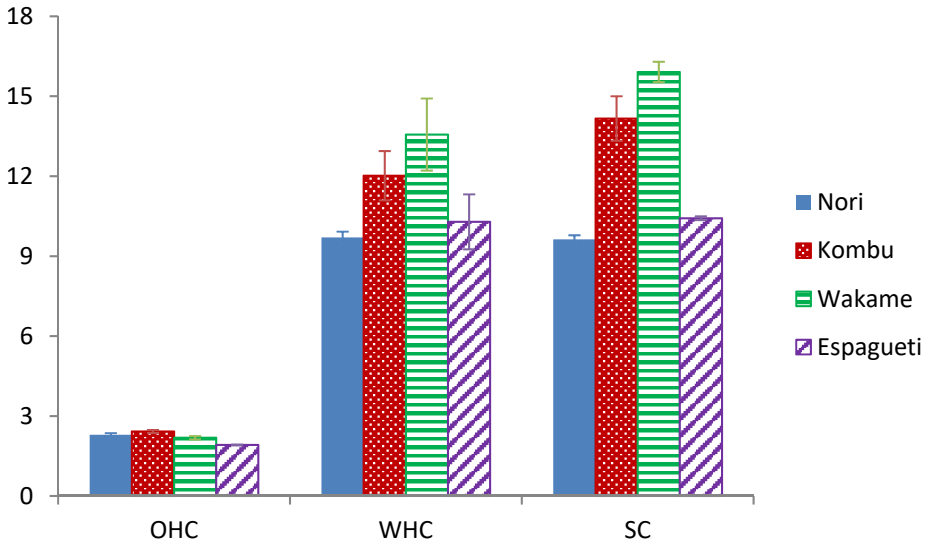
701

702 **Fig. 3.**

703

704

705



706

707 **Fig. 4.**

708

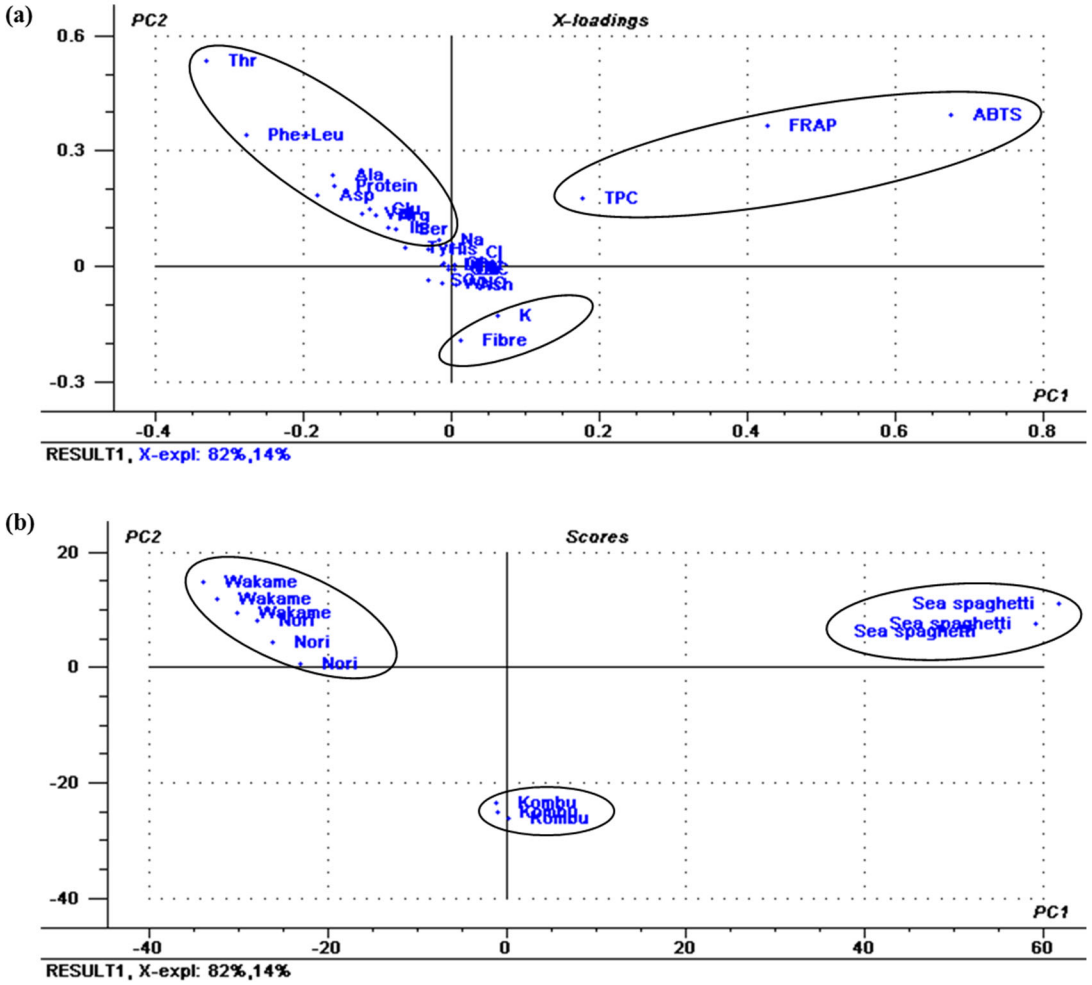

(a)
X-loadings
PC2
PC1
Thr
Phe+Leu
Ala
Protein
Asp
Fiber
Na
Cl
K
Fibre
FRAP
ABTS
TPC
RESULT1, X-expl: 82%,14%
(b)
Scores
PC2
PC1
Wakame
Wakame
Wakame
Nori
Nori
Nori
Sea spaghetti
Sea spaghetti
Sea spaghetti
Kombu
Kombu
Kombu
RESULT1, X-expl: 82%,14%

Fig. 5.