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Proximate composition of several macroalgae from the coast of Salinas Bay, Ecuador

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Abstract: Seaweeds are accessible and important marine organisms found in coastal zones, which have shown their nutritive potential as food or additive. These organisms are relatively abundant in the coastline of Ecuador, but their biochemical composition has not been adequately studied. Therefore, the aim of this research was to evaluate the proximate composition of seven seaweeds (four red, two brown, and one green) collected from Salinas Bay, Ecuador, as a contribution to the knowledge of the nutritional potential of these organisms that belong to this region. Moisture, ash, fat, fiber and protein contents were determined by standard protocols, while carbohydrates were obtained by difference. Energetic content (or caloric profile) was calculated according to the contributions of macromolecules (fats, proteins and carbohydrates). The parameters in highest proportion found in all species were carbohydrates (32.2-45.5 %) and minerals (or ash, 25.8-36.7 %), which play a significant role in human nutrition and the food industry. Furthermore, protein, fiber, and fat contents were relatively low, with values around 4.7-8.0 %, 0.9-5.0 %, and 0.3-3.0 %, respectively, indicating these organisms are a good option as healthy food. According to statistical analysis (ANOVA), each nutritive parameter was significantly different among the species ($P < 0.05$). Results indicate edible seaweeds from Ecuador have potential as nutritious food that could offer between 1 500-2 000 kcal kg^{-1}, which is higher than many common vegetables.

Key words: seaweeds; nutritional profile; seafood; Rhodophyte; Phaeophyte; Chlorophyte.

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"Seaweeds" is the common name of benthic marine macroalgae that encompass a diversity of photosynthetic organisms abundant in all coastal environments around the world, including tropical, temperate and polar environments (Roesijadi, Jones, Snowden-Swan, & Zhu, 2010; Mouritsen, 2013; Hurd, Harrison, Bischof, & Lobban, 2014). They have been considered as a primitive type of plant from shallow waters (Manivannan, Thirumaran, Karthikai, Anantharaman, & Balasubramanian, 2009), due to their similitudes with terrestrial vegetation; however, there are several differences that separate them from the Plantae kingdom and incorporate them into the Protista kingdom (Radulovich, Umanzor, & Cabrera, 2013). Seaweeds are classified into three main groups based on their pigmentation and other characteristics, as red (Rhodophyceae), brown (Phaeophyceae), and green (Chlorophyceae)

(Chapman & Chapman, 1980), being this last group the one with the closest relationship to higher plants (Roesijadi et al., 2010).

Fresh and dried seaweeds have been used as food since ancestral times, especially by people living in coastal areas (Narasimman & Murugaiyan, 2012; Mouritsen, 2013; Radulovich et al., 2013; Evans & Critchley, 2014; Buschmann et al., 2017), reason for which their production (farming or mariculture) for human consumption has increased in the last decades (Titlyanov & Titlyanova, 2010; Hurd et al., 2014; Buschmann et al., 2017; O'Connor, 2017; Qin, 2018). In addition, the growing interest in seaweeds is due to the fact that they are a source of several polysaccharides known as phycocolloids, such as agar, alginate, and carrageenan extracts, which are widely used in food and pharmaceutical industries as thickening and gelling agents (Tori, 2014; Mwalugha, Wakibia, Kenji, & Mwasaru, 2015; Porse & Rudolph, 2017).

Regarding the nutritional contribution of seaweeds, recent studies still demonstrate that they are low calorie foods with high contents of vitamins, minerals, proteins and carbohydrates (Rodrigues et al., 2015; Bhuiyan, Qureshi, Mustafa, AftabUddin, & Momin, 2016; Sivaramakrishnan et al., 2017). Nevertheless, nutritional properties of seaweeds are not completely known yet, because different characteristics, such as species, geographical area, season, among others, could affect their chemical and nutritional composition.

The coastal zone of Ecuador seems to be a source of a great diversity of macroalgae, being Salinas Bay the region that has exhibited a major variety of species (Rubira-Carvache, 2012). Consequently, the nutritional properties of these Ecuadorian edible organisms should be known; this could offer them significant aggregated value, and increase their production and consumption. For this reason, the aim of this work was to evaluate the proximate composition of seven seaweeds collected in the coast of Salinas Bay, Ecuador, contributing to the knowledge of the nutritional value of these marine organisms.

MATERIALS AND METHODS

Collection of samples: Seaweed samples were collected in the coast of Salinas Bay (2°15'0" S & 80°56'0" W), located in the west coast of Santa Elena Province, Ecuador. These algae were taxonomically identified by the biologists Teodoro Cruz Jaime and Raúl Rincones (BIORMA Aquaculture CA) and subjected to a cleaning process with abundant distillated water. Excess water was allowed to drip off under sunlight; then the algae was dried under shade at room temperature for 96 h, and subsequently dried in a stove (Memmert SNB 400 with air flow) at 40 °C for 24 h. Afterwards, the dried seaweeds were pulverized with a grinder (Lab. Mill serial No. 56969, Type AR 400 Erweka®, Germany), hermetically sealed in Ziploc bags and kept at room temperature until further analyses.

Four of the seven species collected, such as *Acanthophora spicifera* (M. Vahl) Børgesen 1910 (Rhodomelaceae), *Centroceras clavulatum* (C. Agardh) Montagne 1846 (Ceramiaceae), *Hypnea spinella* (C. Agardh) Kützing 1847 (Cystocloniaceae) and *Kappaphycus alvarezii* (Doty) Doty ex P.C.Silva 1996 (Solieriaceae), are red seaweeds (Rhodophyta); two species, *Padina pavonica* (Linnaeus) Thivy 1960 (Dictyotaceae) and *Spatoglossum scroederi* (C. Agardh) Kützing 1859 (Dictyotaceae), are brown seaweeds (Ochrophyta); and the last species, *Ulva lactuca* Linnaeus 1753 (Ulvaceae), is a green seaweed (Chlorophyta) (Guiry & Guiry, 2018). Samples of the different species were deposited in the Pharmacy Pilot Plant of *Universidad Técnica de Machala* with the records PPFAS022, PPFCC023, PPFHS024, PPFKA025, PPFPP026, PPFSS027 and PPFUL028, respectively.

Proximate analysis: Moisture and ash contents of dried seaweeds were obtained by gravimetric determinations according to World Health Organization (1998), using an oven at 105 °C and a furnace at 750 °C, respectively. Both analyses were carried out at Pharmacy Pilot Plant, *Universidad Técnica de Machala*, Ecuador. Crude lipid, fiber and protein contents were determined using the methodology reported by Chen, Liu, Zhang, Chen, & Wang (2012), the NTE INEN-ISO 6865 protocol (INEN, 2014), and Kjeldahl (Avilés, 2002), respectively. These last analyses were performed at "Centro de Investigaciones Biotecnológicas del Ecuador", *Escuela Superior Politécnica del Litoral*. Carbohydrates content was calculated by difference as:

$$\%_{\text{Carbohydrate}} = 100 - (\%_{\text{Moisture}} + \%_{\text{Ash}} + \%_{\text{Fat}} + \%_{\text{Fiber}} + \%_{\text{Protein}}) \qquad \text{(Eq. 1)}$$

While the caloric energy of seaweeds was calculated as:

$$\text{Energy} = (9 \text{ kcal g}^{-1} \times \%_{\text{Fat}}) + (4 \text{ kcal g}^{-1} \times \%_{\text{Protein}}) + (4 \text{ kcal g}^{-1} \times \%_{\text{Carbohydrates}}) \qquad \text{(Eq. 2)}$$

All data was expressed in terms of mean ± standard deviation. To calculate mean percentage and standard deviation, Statistical Package for Social Science software (SPSS) Version 23.0 for windows (IBM Corp. Released) was used. To determine whether there were any differences amongst the means, one way Analysis of Variance (ANOVA) and Duncan's multiple range tests were applied to the results and P values < 0.05 were considered significant. Test of normality was previously applied to the dates, which showed being approximately normal.

RESULTS

Table 1 shows the proximate composition of the dried seaweeds collected from Santa Elena, Ecuador. The water content of the seaweeds ranged from 90.74 to 95.97 %, which indicates that the dry weight was lower than 10 % of the fresh organisms. A higher water content implies a lower dry biomass. The lowest water content was found for *Acanthophora spicifera* (90.74 %), followed by *Hynea spinella* (90.89 %). While the highest content of water was found for *Kappaphycus alvarezii* (95.97 %), followed by *Ulva lactuca* (92.31 %).

The brown seaweed *Spatoglossum schroederi* showed the lowest value of moisture (12.84 %) after drying, while the rest of the organisms showed around 20 % of moisture (18.61-23.54 %). Ash content ranged from 25.49 to 36.69 %, being the brown seaweed *Padina pavonica* and the red seaweed *Centroceras clavulatum* the ones which showed the lowest and the highest values, respectively. Fat content oscillated between 0.33 and 3.06 %, being *U. lactuca* and *S. schroederi* the species that exhibited the lowest and highest content of fat, respectively. The highest protein content (8.02 %) was found in the red seaweed *H. spinella*, and the lowest value (4.78 %) was found in the red seaweed *C. clavulatum*, while the rest of the organisms showed protein contents that oscillated around 4.86-5.54 %. Fiber content ranged between 0.96 % and 4.94 %. *P. pavonica* was found to have the highest value, followed by *H. spinella*, and *S. schroederi*, while *U. lactuca* showed the lowest.

Carbohydrate contents were between 32.24 and 45.52 %, *C. clavulatum* and *U. lactuca* showed the lowest and the highest values, respectively. Finally, the caloric energy of seaweeds ranged from 1 547 kcal kg^{-1} (for *C. clavulatum*) to 2 085 kcal kg^{-1} (for *S. schroederi*). According to ANOVA (Table 2), proximate compositions were significantly different among the species (P < 0.05) with some exceptions. Duncan's multiple range tests indicated five, six or seven homogeneous sub-conjuncts among different parameters.

DISCUSSION

Fresh seaweeds naturally contain 80 to 90 % of water (Fontaine & Bonilla, 1978), while Ecuadorian fresh seaweeds contained more than 90 % (Table 1). These values are similar or slightly higher than those reported in the literature for other seaweeds (Hussain

TABLE 1
Proximate composition, expressed as percentage (%), of seaweeds collected in Bay of Salinas, Santa Elena, Ecuador

Parameter	*A. spicifera*	*C. clavulatum*	*H. spinella*	*K. alvarezii*	*P. pavonica*	*S. schroederi*	*U. lactuca*
Water*	90.74	91.89	90.89	95.97	91.57	91.60	92.31
Moisture**	18.84 ± 0.13^{a}	23.54 ± 0.22^{b}	18.61 ± 0.11^{a}	22.77 ± 0.15^{c}	20.47 ± 0.26^{d}	12.84 ± 0.18^{e}	20.67 ± 0.27^{d}
Ash**	28.38 ± 0.39^{a}	36.69 ± 0.22^{b}	33.07 ± 0.12^{c}	27.49 ± 0.15^{d}	24.85 ± 0.26^{e}	34.58 ± 0.20^{f}	26.99 ± 0.26^{d}
Fat**	0.55 ± 0.01^{a}	0.75 ± 0.02^{b}	1.44 ± 0.01^{c}	0.57 ± 0.01^{a}	0.83 ± 0.01^{d}	3.07 ± 0.02^{e}	0.33 ± 0.01^{f}
Protein**	5.07 ± 0.01^{a}	4.78 ± 0.02^{b}	8.02 ± 0.01^{c}	4.86 ± 0.01^{d}	5.53 ± 0.01^{e}	5.21 ± 0.02^{f}	5.54 ± 0.02^{e}
Fiber**	2.42 ± 0.24^{a}	2.02 ± 0.11^{b}	4.41 ± 0.19^{c}	3.18 ± 0.11^{d}	4.94 ± 0.16^{e}	4.28 ± 0.20^{c}	0.96 ± 0.03^{f}
Carbohydrate***	44.76 ± 0.01^{a}	32.24 ± 0.12^{b}	34.46 ± 0.18^{c}	41.15 ± 0.11^{d}	43.39 ± 0.16^{e}	40.04 ± 0.21^{f}	45.52 ± 0.04^{g}
Energy*** (kcal kg^{-1})	2 041.65 ± 0.66	1 547.26 ± 2.99	1 828.19 ± 5.94	1 890.42 ± 2.84	2 030.61 ± 4.91	2 085.26 ± 6.32	2 071.45 ± 0.15

* On a wet weight basis.
** Values are means of two replicates ± standard deviations on a dry weight basis.
*** Calculated values.
Values with different letters within a row are significantly different ($P \leq 0.05$) according to ANOVA.

et al., 2009; Ahmad, Sulaiman, Saimon, Fook, & Matanjun, 2012; Sivaramakrishnan et al., 2017). Furthermore, after drying, the seaweeds still had relatively high residual moisture content, approximately 20 % (on a dry weight basis), which indicates that the method used to dry these organisms was possibly not optimal. Other authors have reported minor residual moisture in proximate analysis of seaweeds using methods such as oven drying at 60 °C or freeze-dry (Rohani-Ghadikolaei, Abdulalian, & Ng, 2012; Rodrigues et al., 2015; Bhuiyan et al., 2016). Removal of water from seaweeds is a necessary step in maintaining their quality as a food, since it would impede the chemical and biochemical reactions of degradation and delays the growth of microorganisms that can deteriorate them (Coenders, 1996).

On the other hand, Fontaine & Bonilla (1978) have indicated that seaweeds can contain around 40 % of carbohydrates, from 1 to 3 % of lipids, and from 3 to 38 % of minerals (ash). Results of the proximate composition of Ecuadorian seaweeds (Table 1) are consistent with that information; for instance, carbohydrates were the parameter found in highest percentage (30-45 %), followed by ash content (25-37 %). However, the specie *C. clavulatum* showed a higher content of ash (36.69 %) followed by carbohydrates (32.24 %). Possibly, this seaweed requires a mayor content of minerals for structural and ecological interactions, while its lower carbohydrate content could be due to the fact that it was in a different stage of growth respect to the other species (Marinho-Soriano, Fonseca, Carneiro, & Moreira, 2006).

Other studies have reported high content of carbohydrate and/or ash in seaweeds (Nguyen, Ueng, & Tsai, 2011; Rohani-Ghadikolaei et al., 2012; Gokulakrishnan, Raja, Sattanathan, & Subramanian, 2015; Bhuiyan et al., 2016; Sivaramakrishnan et al., 2017), which suggests these two parameters are important in the composition and biochemical functions of the seaweeds. Moreover, the high content of carbohydrates suggests these organisms could be an important source of phycocolloids for industrial uses. In terms of fat content, the values found

TABLE 2
One-way ANOVA between seaweeds and nutritional parameters

Source of variation		SS	df	MS	F	P-value
Moisture	Between groups	149.51	6	24.92	631.32	0.000
	Within groups	0.28	7	0.04		
	Total	149.79	13			
Ash	Between groups	237.93	6	39.65	686.79	0.000
	Within groups	0.40	7	0.06		
	Total	238.33	13			
Protein	Between groups	15.01	6	2.50	15 922.74	0.000
	Within groups	0.00	7	0.00		
	Total	15.01	13			
Fat	Between groups	10.71	6	1.78	15 612.92	0.000
	Within groups	0.00	7	0.00		
	Total	10.71	13			
Fiber	Between groups	25.34	6	4.22	163.39	0.000
	Within groups	0.18	7	0.03		
	Total	25.52	13			
Carbohydrate	Between groups	312.92	6	52.15	2 876.26	0.000
	Within groups	0.13	7	0.02		
	Total	313.05	13			

in five of these organisms were relatively very low (< 1 %), indicating that these seaweeds are an ideal food choice for people that require a low-fat or fat-free diet. Lastly, protein and fiber contents were moderately low compared to known vegetables and other macroalgae (Rodrigues et al., 2015; Bhuiyan et al., 2016; Sivaramakrishnan et al., 2017).

Although the proximate compositions of the seaweeds were very similar, statistical analysis indicated significant differences among the species. Each Ecuadorian macroalgae exhibited a slightly different proximate composition to that reported in other studies. For instance, *A. spicifera* showed minor ash, protein and lipid contents, and higher carbohydrate content than the values reported by Mohammadi, Tajik, & Hajeb (2013). A similar case was found for *C. clavulatum* that showed lower lipid and protein contents, and higher carbohydrate content than the values indicated by Diniz, Barbarino, & Lourenço (2012).

Fat, protein and ash contents of *H. spinella* were lower than those reported by Viera et al. (2005), while carbohydrate content was higher. The proximate composition found for *K. alvarezii* in this study was similar to the results found by Abirami & Kowsalya (2011), although different to that reported by Kumar, Ganesan, & Subba (2015), who demonstrated the seasonal changes in nutritional composition of this species in other region. Manivannan et al. (2009) and Tabarsa, Rezaei, Ramezanpour, Waaland, & Rabiei (2012) reported values of protein, fiber, ash and lipid for *P. pavonica* higher than the values found in this research. The content of fat and ash found in *S. schroederi* were higher than values reported for other species belongs to *Spatoglossum* genus (Mwalugha et al., 2015), while fiber and protein contents found in this study were lower. Ash content found for Ecuadorian *U. lactuca* was higher than that reported by Abirami & Kowsalya (2011) and Mwalugha et al. (2015); while, the other nutritional parameters, such as carbohydrate, protein, fiber and fat contents, were found in minor proportion in this study. The results obtained confirm the relationship between the chemical composition of the seaweeds and differences in the geographical area, environmental conditions and seasons where they grow, among other aspects.

Caloric profiles of the seaweeds (energy offered by macromolecules such as carbohydrates, fat and protein) were similar to those reported by Admassu, Abera, Abraha, Yang, & Zhao (2018) for the commercial edible seaweed *Porphyra* spp.; in the case of Ecuadorian seaweeds, the major caloric contribution is due to the carbohydrate content. In conclusion, seaweeds from the coast of Ecuador have comparable nutritional value to that of terrestrial vegetables and other seaweeds around the world. These showed a low content of lipids and an important fraction of fiber, protein and carbohydrates, reason for which they could be considered a healthy food with low caloric profiles. In this sense, farming of seaweeds might become an economic option for people from this region, contributing not only with a new nutritive food, but also improving the marine ecosystems.

Ethical statement: authors declare that they all agree with this publication and made significant contributions; that there is no conflict of interest of any kind; and that we followed all pertinent ethical and legal procedures and requirements. A signed document has been filed in the journal archives.

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RESUMEN

Composición proximal de varias macroalgas de la costa de Bahía Salinas, Ecuador. Las macroalgas marinas son organismos accesibles e importantes de las zonas costeras, los cuales han mostrado su potencial como alimentos o aditivos nutritivos. En la línea costera de Ecuador estos organismos son relativamente abundantes, pero su composición bioquímica no ha sido estudiada adecuadamente. En consecuencia, el objetivo de esta investigación fue evaluar la composición proximal de siete especies de macroalgas (cuatro rojas, dos pardas y una verde) que fueron recolectadas en la Bahía de Salinas, Ecuador, como una contribución al conocimiento del potencial nutricional de estos organismos pertenecientes a esta región. Los contenidos de humedad, cenizas, grasa, fibra y proteínas fueron determinadas mediante protocolos estándares, mientras que el contenido de carbohidrato fue obtenido por diferencia. El contenido energético (o perfil calórico) de las macroalgas fue calculado de acuerdo con las contribuciones de las macromoléculas (grasas, proteínas y carbohidratos). Los parámetros encontrados en mayor proporción en todas las especies fueron: carbohidratos (32.2-45.5 %) y minerales (o cenizas, 25.8-36.7 %), los cuales tienen importancia en la nutrición humana y la industria alimentaria. Además, los contenidos de proteína, fibra y grasa fueron relativamente bajos, encontrando valores alrededor de 4.7-8.0, 0.9-5.0, y 0.3-3.0 %, respectivamente, indicando que estos organismos son una buena y saludable opción como alimento. De acuerdo con el análisis estadístico (ANOVA), cada parámetro nutritivo fue significativamente diferente entre especies ($P < 0.05$). Los resultados indican que las algas comestibles de Ecuador tienen potencial como alimento nutritivo que pueden ofrecer entre 1 500-2 000 kcal kg^{-1}, un aporte energético un poco mayor que muchos vegetales comunes.

Palabras clave: macroalgas; perfil nutritivo; comida marina; Rhodophyte; Phaeophyte; Chlorophyte.

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