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Proximate biochemical composition and mineral content of edible species from the genus *Cystoseira* in Portugal

Abstract: Macroalgae are valuable resources for human consumption in many countries. This work reports for the first time a comparative evaluation of the nutritional properties of five edible macroalgae from the genus *Cystoseira*, namely *C. humilis*, *C. tamariscifolia, C. nodicaulis, C. compressa* and *C. baccata*. For this purpose, their proximate composition was determined in terms of moisture, ash, and total contents of protein, lipids, carbohydrates and mineral profile. *Cystoseira tamariscifolia* and *C. baccata* were the species that in general had the higher ash, protein and lipid contents, while the highest levels of moisture and total carbohydrates were detected in *C. nodicaulis* and *C. compressa*. *Cystoseira* species had also high amounts of minerals, especially of potassium, calcium and iron, and a favorable Na/K ratio. The present study shows that *Cystoseira* has a balanced nutritional composition, suitable for human consumption, and that its intake can contribute to a healthy and well-balanced diet.

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Introduction

It is estimated that the world's population will grow to nine billion by mid-century, putting substantial demands on the planet's food supply. Macroalgae (also known as "seaweeds") are major coastal resources that are valuable for human consumption in many countries. Edible macroalgae are widely consumed in Asia, but the demand has grown worldwide, especially in the USA and Europe. Seaweeds are a highly nutritive food that additionally can be eaten in raw salads, soups, cookies, meals, and condiments (Aguilera-Morales et al. 2005). The nutritional value ascribed to macroalgae along with their non-animal nature makes them particularly appropriate for use in the food and additive industries (Lordan et al. 2011). However, compared to land plants, the chemical composition of macroalgae has been poorly investigated and most of the available information refers to species traditionally consumed in Japanese cuisine. There has also been a growing interest in the mineral content of macroalgae, which is higher than that of many terrestrial plants (Tabarsa et al. 2012). *Cystoseira* (C. Agardh) is one of the most widely distributed genera of the class Phaeophyceae and is abundant not only on European coasts, but also in the Pacific and Indian Oceans (Valls and Piovetti 1995). Several species belonging to the Phaeophyceae, such as *Laminaria japonica* (Areschoug) and *Sargassum naozhouense* (C.K. Tseng and Lu Baoren) (Peng et al. 2013, Patra et al. 2015), among many others, are traditionally used as food. Although *Cystoseira* species are not commonly used as food, they are defined as edible and are potential candidates for food products, nutraceutical and pharmaceutical preparations (Andrade et al. 2013) and thus could be considered as alternative sources of nutritional elements. Therefore, the focus of the present study was to evaluate the proximate composition and mineral content of five species of brown

macroalgae belonging to the genus, namely *Cystoseira humilis* Schousboe ex Kützing, *C. tamariscifolia* (Hudson) Papenfuss, *C. nodicaulis* (Withering) M. Roberts, *C. compressa* (Esper) Gerloff and Nizamuddin and *C. baccata* (S.G. Gmelin) P.C. Silva, as well as to consider and discuss the use of these macroalgae as food and/or incorporated in supplements. To the best of the authors' knowledge this is the first report on the proximate composition and mineral contents of these *Cystoseira* species.

Materials and methods

Algal biomass sampling and preparation

Cystoseira humilis, *C. compressa*, *C. tamariscifolia*, *C. nodicaulis* and *C. baccata* were collected in the middle/lower intertidal areas of the Algarve (Albufeira and Odeceixe, Portugal) coast, in May 2010, during low tide (Table 1). Specimens were identified by Dr. Aschwin Engelen (Centre of Marine Sciences, University of Algarve, Portugal) and Dr. Javier Cremades Ugarte (Faculty of Sciences, University of A Coruña). Voucher specimens of *C. compressa* (code number MB004), *C. humilis* (code number MB007), *C. tamariscifolia* (code number MB016), *C. nodicaulis* (code number MB014-2), and *C. baccata* (code number MB001) are deposited at the Centre of Marine Sciences, University of Algarve. Individual thalli were washed thoroughly with freshwater upon arrival in the laboratory to remove epiphytes and washed again with distilled water. In order to have enough biomass for the determination of the proximate composition and minerals, six replicates were prepared by combining two to three individual thalli of a given species. Each replicate sample was assessed for moisture content while fresh and was then oven-dried, ground into a fine powder and stored in tightly closed plastic bags containing silica gel until analysis.

Proximate biochemical analysis

For the determination of the proximate composition, six replicates were prepared as described in the previous section. Moisture was determined by drying the replicates at 95°C until constant weight as described by the AOAC method (AOAC 1995). Ash content was measured by weight difference before and after 5 h of incineration in a muffle furnace at 525°C. Total nitrogen content of dried samples was determined using a Vario EL III elemental analyzer (Elementar, Hanau, Germany), and the protein content was estimated by multiplying the total nitrogen content by a nitrogen conversion factor of 6.25 (Lourenço et al. 2002). Total lipid content was determined by a modified method of Bligh and Dyer (Pereira et al. 2013). Carbohydrate content was assumed to be the remaining biomass and was calculated from the difference between 100% and the summed contents of ash, protein and lipids (Marinho-Soriano et al. 2006).

Mineral content

Mineral elements were analyzed by atomic absorption spectrometry (AAS). Approximately 300 mg of the dried powder from three of the replicate samples for each species were digested by microwave (Milestone Ethos Touch, Sorisole, Italy) in high-pressure Teflon vessels. The digestion was made using 6 ml of Fluka supra-pure HNO_3 (65%), 1 ml of Riedel-Dehaen p.a. $HClO_4$ (70%) and 1 ml of Merck supra-pure H_2O_2 (30%) (ETHOS PLUS, 2001). A procedural blank was prepared and included in each digestion batch of 10 samples. Calcium, magnesium, sodium, potassium, iron, manganese and zinc were analyzed by flame AAS with an air-acetylene flame in an atomic absorption spectrometer (GBC Avanta Sigma, Victoria, Australia). The accuracy of the analytical procedure was assessed by the analysis of certified reference material (BCR-60 – aquatic plant *Lagarosiphon major*, from the Institute for Reference

Table 1: Date and location of sample collection of different *Cystoseira* species from Portugal.

	Date	GPS coordinates	Location (beach, county)	Voucher specimen code number
C. humilis	07-05-2010	37°4′36.19″N 8°18′36.49″W	Manuel Lourenço, Albufeira	MB007
C. compressa	08-05-2010	37°4′35.83″N 8°16′33.74″W	Arrifes, Albufeira	MB004
C. tamariscifolia	07-05-2010	37°5′24.31″N 8°11′9.72″W	Olhos de Água, Albufeira	MB016
C. nodicaulis	07-05-2010	37°4′36.19″N 8°18′36.49″W	Manuel Lourenço, Albufeira	MB014-2
C. baccata	11-05-2010	37°26′41.48″N 8°48′3.90″W	Odeceixe, Aljezur	MB001

Materials and Measurements, JRC-IRMM, Belgium). Procedural blanks always accounted for <1% of the metal concentrations in samples. Values were expressed as g kg^{-1} dry weight (DW) for Ca, Mg, Na and K or mg kg^{-1} DW for Fe, Mn and Zn.

Statistical analysis

Biological replicates were collected on a given date and location (Table 1) and were used for the determination of the proximate (n=6) and mineral composition (n=3). Significant differences were assessed by analysis of variance (ANOVA) and significance between means was analyzed by the Tukey HSD test (p<0.05). SPSS statistical package for Windows (release 15.0, SPSS Inc.) was used.

Results

The present study aimed to evaluate the proximate composition of different *Cystoseira* species (namely *C. humilis*, *C. compressa*, *C. tamariscifolia*, *C. nodicaulis* and *C. baccata*) collected on the Algarve coast, Portugal. Results are summarized in Table 2. The moisture content ranged from 49% of wet weight (WW) in *C. tamariscifolia* to 63% in *C. compressa*, while the ash levels varied from 7% in *C. compressa* to 24% in *C. tamariscifolia*. *Cystoseira tamariscifolia* also presented the lowest total carbohydrate (54%) and the highest protein (13%) contents. Conversely, *C. nodicaulis*, along with *C. compressa*, had the highest total carbohydrate levels (73%) and low protein contents (9–10%). The highest concentration of total lipids was 11% in *C. baccata*, followed by *C. tamariscifolia* (10%) and the lowest value was observed in *C. nodicaulis* (4%, Table 2).

The mineral content of the samples was also evaluated and results are given in Table 3. The total of all macrominerals, expressed as the sum of K, Na, Ca and Mg, ranged from 40 g kg^{-1} in *C. baccata* to 101 g kg^{-1} in *C. compressa*.

However, in terms of microminerals, *C. tamariscifolia* had the highest (1013 mg kg^{-1}) and *C. compressa* the lowest (173 mg kg^{-1}) content. Potassium was the most abundant macromineral in *C. compressa*, *C. humilis* and *C. baccata* (60, 30 and 22 g kg^{-1}, respectively). Sodium content ranged from 8 g kg^{-1} in *C. humilis* to 16 g kg^{-1} in *C. tamariscifolia*. The Na/K ratios varied from 0.15 in *C. compressa* and 0.97 in *C. tamariscifolia* (Table 3). In *C. tamariscifolia* and *C. nodicaulis*, calcium was the most abundant macromineral (around 26 g kg^{-1} in both species). Magnesium concentration varied from 5.7 g kg^{-1} in *C. nodicaulis* to 19 g kg^{-1} in *C. compressa*.

Among the microminerals detected, iron was the most abundant ranging from 110 mg kg^{-1} in *C. baccata* to 508 mg kg^{-1} in *C. tamariscifolia*. *Cystoseira tamariscifolia* also had the highest content of manganese (398 mg kg^{-1}), while the lowest manganese content was observed in *C. compressa* (14 mg kg^{-1}), which also contained the lowest amount of zinc (9.4 mg kg^{-1}). The highest content of zinc was found in *C. nodicaulis* (114 mg kg^{-1}), although *C. tamariscifolia* and *C. baccata* also had values above 100 mg kg^{-1} (Table 3).

Discussion

This study reports for the first time the proximate composition and mineral profiles of five species of *Cystoseira* found on the coast of Algarve (Portugal), namely *C. humilis*, *C. tamariscifolia*, *C. nodicaulis*, *C. compressa* and *C. baccata*. The first parameter assessed was the moisture content of fresh specimens. As found in most brown macroalgae, the moisture values for fresh specimens were high. For example, the moisture determined for *C. compressa* was 63%, which is similar to the value reported for *Sargassum* (C. Agardh) (Holdt and Kraan 2011).

Most ash contents obtained for *Cystoseira* are in agreement with published values for other Phaeophyceae, such as those of *Ascophyllum* (Stackhouse) (18–27%), *Fucus* (Linnaeus) (19–30%) and *Sargassum* (14–44%). The only

Table 2: Proximate composition of different *Cystoseira* species from Portugal, including moisture (% of wet weight), ash, total protein, total lipids, carbohydrates (% of dry weight).

	C. humilis	*C. compressa*	*C. tamariscifolia*	*C. nodicaulis*	*C. baccata*
Moisture	57.06[b]	63.05[a]	48.99[d]	58.95[b]	51.43[c]
Ash	20.35[a,b]	7.30[d]	23.85[a]	13.45[c]	19.10[b]
Protein	10.34[b]	10.16[b]	12.52[a]	9.20[c]	12.46[a]
Lipids	5.22[c]	9.45[b]	9.57[b]	4.31[d]	10.92[a]
Carbohydrates	64.09	73.09	54.06	73.04	57.52

Values are mean (n=6). Different superscript letters indicate that, for each variable, differences between species are significant at p=0.05.

Table 3: Mineral content of different *Cystoseira* species from Portugal.

Mineral	Symbol	C. humilis	C. compressa	C. tamariscifolia	C. nodicaulis	C. baccata
Expressed as g kg^{-1} dry biomass						
Calcium	Ca	23.70[b]	13.08[c]	25.82[a]	26.50[a]	9.43[d]
Magnesium	Mg	12.90[b]	18.53[a]	6.56[c]	5.69[d]	6.78[c]
Sodium	Na	7.56[e]	8.94[d]	16.36[a]	11.61[b]	10.81[c]
Potassium	K	29.78[b]	60.02[a]	16.87[d]	15.98[e]	22.10[c]
Na/K ratio		0.25	0.15	0.97	0.73	0.49
Total		73.94	100.57	65.61	59.78	39.69
Expressed as mg kg^{-1} dry biomass						
Iron	Fe	171.88[c]	149.74[d]	508.06[a]	413.14[b]	109.59[e]
Manganese	Mn	152.18[c]	14.27[e]	398.46[a]	193.54[b]	108.35[d]
Zinc	Zn	41.16[c]	9.43[d]	105.99[b]	113.82[a]	106.73[b]
Total		365.22	173.44	1012.51	720.50	324.67

Values are means (n=3). Different superscript letters indicate that, for each variable, differences between species are significant at p=0.05.

exception was the ash content for *C. compressa* (7%), which is lower than the usual range (Holdt and Kraan 2011).

The protein values obtained in this study (9–13% DW) are of the same order as those reported in previous studies of brown algae. According to the review by Holdt and Kraan (2011), the protein content of *Ascophyllum* can range from 1.2 to 12%, and that of *Sargassum* from 9 to 20%. The protein content is often lower in brown macroalgae than in green and red macroalgae (Holdt and Kraan 2011).

In general, macroalgae have low lipid contents, usually below 5% of dry matter (Burtin 2003). However, *C. compressa, C. tamariscifolia* and *C. baccata* had total lipid contents higher than 9.4%. Gosch et al. (2012) showed that brown macroalgae typically have the highest content of total lipids amongst seaweeds. Some species have values of total lipids higher than 15%. This is particularly true in members of the order Dictyotales, which consistently display total lipid contents between 11 and 20% DW (McDermid and Stuercke 2003). Our results are in accordance with these data, showing that the closely related *Cystoseira* (Fucales) algae also contain relatively high lipid contents. The fatty acid profiles of these five species of *Cystoseira* have been reported previously (Vizetto-Duarte et al. 2015) and showed that their polyunsaturated fatty acids (PUFA) corresponded to 29–46% of the total fatty acids detected. In addition, *Cystoseira* species have a high nutritional value due to their high PUFA vs. saturated fatty acids (SFA) ratios and low n-6 PUFA/n-3 PUFA ratios (Vizetto-Duarte et al. 2015).

In this study, the carbohydrate content varied from 42 to 73% DW in *Cystoseira* species. Hadj Ammar et al. (2015) showed lower total carbohydrate contents (13–45% DW) for *C. compressa*, *C. sedoides* and *C. crinita* collected in June in Tunisia. However, other brown algae collected throughout the year, such as *Sargassum vulgare*, have previously been reported with a carbohydrate content of 68% DW (Marinho-Soriano et al. 2006). Variation in temperature, salinity and solar radiation among the sites of collection might explain the aforementioned differences, as all of these environmental factors seem to have a significant correlation with carbohydrate content (Marinho-Soriano et al. 2006). Moreover, carbohydrate content might also vary with the physiological state (e.g. developmental stage; Holdt and Kraan 2011) of the alga. Previous studies have suggested an inverse relationship between carbohydrates and proteins in macroalgae (Mouradi-Givernaud et al. 1993, Marinho-Soriano et al. 2006, Kumar et al. 2015), which is also observed in our samples.

Macroalgae are known to have a high mineral content, usually higher than that of most terrestrial plants (Ortega-Calvo et al. 1993). In fact, edible seaweeds are considered to be valuable nutritional resources due to their high content of several essential minerals (Bocanegra et al. 2009). Potassium and sodium values of the macroalgae studied here were higher than those reported for land vegetables, such as lettuce and spinach (USDA 2015). Nevertheless, the Na/K ratios were below 1.0 in all the species of *Cystoseira* studied (0.15–0.97, Table 3), which is interesting from a nutritional point of view, as diets with a high Na/K ratio have been associated with the incidence of hypertension (Taboada et al. 2010). For instance, Na/K ratio in sausages is 4.89 (Ortega-Calvo et al. 1993). High values of calcium and magnesium were also observed, as compared with those of land vegetables (USDA 2015). Regarding microminerals, *C. tamariscifolia* and *C. nodicaulis* displayed remarkably high iron contents (>400 mg kg^{-1}) compared with those of other *Cystoseira* species (Table 3) and of commercial seaweeds such as *Fucus*, *Laminaria*, *Undaria*, *Chondrus* and *Porphyra* (33–103 mg kg^{-1}; Rupérez

2002). The same conclusion can be drawn with respect to the iron concentrations usually found in terrestrial vegetables (35–233 mg kg^{-1}; USDA 2015). In a study by Rupérez (2002), *Fucus vesiculosus* contained 55 mg kg^{-1} of Mn and 71 mg kg^{-1} of Zn. The concentrations of these trace elements detected by us (Table 3) also fall within the ranges observed in previous reports on macroalgae, except for *C. compressa* that exhibited lower values. Mineral composition is also known to vary according to seasonal, environmental, geographical and physiological factors (Mabeau and Fleurence 1993).

Taken together, our results indicate that *Cystoseira* has a balanced nutritional composition suitable for human consumption, and that its intake could contribute to a healthy and well-balanced diet. This study showed that species of *Cystoseira* have high ash and carbohydrate contents, and relatively high total lipids compared to other macroalgae. Mineral analysis also showed that these species contain useful amounts of macrominerals and trace elements. Among the nutritional profiles described here for the first time, *C. tamariscifolia* stands out for its high content of ash (and therefore, minerals) and total protein, its high lipid content, and relatively low carbohydrate content. *Cystoseira tamariscifolia* also contains high Ca and was the species with highest micromineral concentrations, especially Fe and Mn. The Na/K ratio of all species was suitable for human diet. Together with recent studies on the edibility of *Cystoseira* and the need for alternative sources of non-animal food, our results show that these species could be used as alternative sources of valuable food products.

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Fernando Albericio is a Research Professor at the University of KwaZulu-Natal in Durban, South Africa and at the University of Barcelona. He holds a PhD in Chemistry from the University of Barcelona (1980). Following his postdoctoral work (1981–1984) at Tufts University, the Université d'Aix-Marseille, and the University of Minnesota, he was appointed Associate Professor at the University of Barcelona. After a few years, he took up the position of Director of Peptide Research at Milligen-Biosearch, Boston, USA (1992–1994), and then re-joined the University of Barcelona in 1995, when he was promoted to Full Professor. He also served as Executive Director of the Barcelona Science Park (2005–2011). He is currently Editor of the *International Journal of Peptide Research and Therapeutics* and a Councilor of the American Peptide Society. Recently, he has been honored with a doctorate *honoris causa* by the Universidad de Buenos Aires (Argentina) and the Vincent du Vigneaud award by the American Peptide Society.

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João Varela is an Assistant Professor at the University of Algarve and Group Leader of the MarBiotech (Marine Biotechnology) research group at the Centre of Marine Sciences (CCMAR). MarBiotech, which has the following research lines (i) searching for novel bioactive compounds in marine organisms, with particular emphasis on microalgae, macroalgae and halophytes; (ii) designing and implementation of biorefineries for the upgrade of algal biomass for biofuel, food and feed production; and (iii) marine organisms (e.g. sea cucumbers and halophytes) as innovative gourmet food.

Graphical abstract

Catarina Vizetto-Duarte, Luísa
Custódio, Luísa Barreira, Manuela
Moreira da Silva, Amélia P. Rauter,
Fernando Albericio and João Varela

**Proximate biochemical composition
and mineral content of edible
species from the genus *Cystoseira*
in Portugal**

Research article: *Cystoseira* are
nutritious brown macroalgae, rich
in lipids and microminerals, such as
iron. They present a favorable Na/K
ratio and a composition suitable for
human consumption, which suggests
that their intake could contribute to a
well-balanced, healthy diet.