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ORIGINAL PAPER

Undervalued Atlantic brown seaweed species (*Cystoseira abies-marina* and *Zonaria tournefortii*): influence of treatment on their nutritional and bioactive potential and bioaccessibility

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Abstract

The brown seaweed species *Cystoseira abies-marina* and *Zonaria tournefortii* are abundant Atlantic resources that remain undervalued. This results from an insufficient knowledge of their nutrients' and bioactive potential. There is also uncertainty regarding the adequate culinary treatment of these seaweeds prior to their consumption. Thus, the current study evaluated the composition, bioactivity, and bioaccessibility of target compounds and bioactivities of these two species as a function of two treatments, simple rehydration and steaming, in comparison to sun-dried seaweed. The proportion of SFA, MUFA, and PUFA differed between species. *C. abies-marina* was richer in PUFA (30–31% vs 20–21%) and *Z. tournefortii* was richer in SFA (53–57% vs 46–47%). Main contributors to ω3 PUFA content were different in each species: alpha-linolenic acid in *C. abies-marina*, 4.5–5.1%, and eicosapentaenoic acid in *Z. tournefortii*, 5.8–6.7%. The sum of Mg and Ca contents in *Z. tournefortii* was two-fold the same sum in the other species. Furthermore, rehydration led to an elemental concentration reduction in most instances. The As content in *C. abies-marina* was very high, ranging between 295 ± 5 mg/kg dw and 369 ± 2 mg/kg dw, in rehydrated and steam-cooked seaweed, respectively. While aqueous extracts of *C. abies-marina* had the highest phenolic contents, 620–1280 mg GAE/100 g dw, aqueous extracts of *Z. tournefortii* contained 170–280 mg GAE/100 g dw. Regarding bioaccessibility, Mg, K, Ca, As, and Cd showed relatively high bioaccessibility levels and it was shown that only a limited part of the original antioxidant activity in both species is bioaccessible.

Keywords *Cystoseira abies-marina* · *Zonaria tournefortii* · Proximate and elemental composition · Fatty acid profile · Bioactivity · Bioaccessibility

Abbreviations

AA Eq	Ascorbic acid equivalent
ABTS	2,2′-Azino-bis(3-ethylbenzothiazoline-6-sulphonic acid)
DPPH	2,2-Diphenyl-1-picrylhydrazyl
FAME	Fatty acid methyl ester
FRAP	Ferric reducing antioxidant power
GAE	Gallic acid equivalent
MUFA	Monounsaturated fatty acid
PUFA	Polyunsaturated fatty acid
SFA	Saturated fatty acid
Trolox Eq	Trolox equivalent
ω3 PUFA	Omega-3 polyunsaturated fatty acid
ω6 PUFA	Omega-6 polyunsaturated fatty acid

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Introduction

While *Cystoseira abies-marina* (S.G. Gmelin) C. Agardh is a brown edible and marketed macroalga (class Phaeophyceae, order Fucales) that is distributed in the Mediterranean, Macaronesian Region and in the coast of Africa,

Zonaria tournefortii (J.V. Lamouroux) Montagne is a brown macroalga (class Phaeophyceae, order Dictyotales) found throughout the Atlantic Ocean [1].

In particular, *C. abies-marina* has been reported as a source of phenolic compounds, encompassing phlorotannins [2]. It is also worth mentioning that this seaweed species has demonstrated a high and selective antiproliferative activity against HeLa cells (IC50—8.8 µg/ml) in an assessment of its pharmacological potential [3]. Regarding *Z. tournefortii*, while a moderate total phenolic content was found by Mekinić et al. [4], another study [5] reported high relative contents of chlorophyll *a*, total carotenoids, total phenolic content, and antioxidant activity in this seaweed species from the Madeira Archipelago when compared with green and red seaweeds of the same region. In spite of these studies, there are still sparse data on antioxidant activity for these seaweed species, being claimed that the *Cystoseira* genus has one of the highest total phenolic levels and antioxidant activities among Phaeophyceae, such as *Bifurcaria bifurcata*, *Fucus ceranoides*, and *Halidrys siliquosa* [6].

The presence of bioactive compounds in the biomass of these two seaweed species as suggested by these few studies has not led to the development of applications until now. Hence, these macroalgae may be considered undervalued. Possible applications may exist in the feed and food sector. Indeed, in general, it has been advocated the existence of extra health benefits from seaweed supplementation alongside a regular diet [7]. The bioactive compounds in seaweed may help to ameliorate digestive health (including dietary fibre), gastrointestinal inflammation (several anti-inflammatory compounds), and other adverse health conditions [8]. However, the development of nutraceutical applications from seaweed biomass has not been particularly successful [7]. Low bioaccessibility—share of the initial component content that is rendered free from the seaweed structure into the gastrointestinal tract [9]—of seaweed components may be a problem [10, 11]. For this reason, it is recommended an evaluation of the bioaccessibility and bioavailability of the algal bioactive compounds [7]. Bioaccessibility studies require appropriate in vitro digestion models that must be optimized in order to simulate human digestion [9, 12].

Moreover, given the high moisture content of seaweed, often exceeding 90% of the wet weight and making biomass highly perishable [13], it is important to dry it for a better preservation [14]. Usually, in the industry, seaweed biomass is dried, sometimes still sun-dried, and stored at a temperature between 0 and 4 °C. For this reason, marketed seaweed is commonly found dry. It may be consumed in this form, but it may also be rehydrated or even cooked (boiling, steaming, etc.). This issue may have an effect on bioactive potential and bioaccessibility, but it is rarely considered in other studies.

Accordingly, this study is directed to the assessment of the composition, bioactive potential, and bioaccessibility of the undervalued brown seaweeds *Cystoseira abies-marina* and *Zonaria tournefortii* as a function of their consumption form, either sun-dried, steam-cooked or simply rehydrated.

Materials and methods

Human and animal rights

Not relevant in this study.

Seaweed collection and treatment

Cystoseira abies-marina and *Zonaria tournefortii*, brown seaweed species from the class Phaeophyceae, were harvested in the mid-north Atlantic island Faial (38° 35′ N latitude), belonging to the Portuguese Archipelago Azores. Both species were harvested in their abundance season; while *C. abies-marina* was harvested in June, *Z. tournefortii* was collected in October. These marketed seaweeds were identified and provided by the Portuguese company seaExpert, sun-dried, packed in black plastic bags, and sent to the Portuguese Institute for the Sea and Atmosphere (IPMA) in Lisbon.

For these seaweed species, two different types of treatment were applied: steam cooking and rehydration. For the former, both seaweed species were steamed during 30 min applying the culinary procedure as usually carried out in the household. For the latter, these seaweed species were rehydrated with water:dry seaweed ratios of 10:1 and 20:1, w/w, for *C. abies-marina* and *Z. tournefortii*, respectively. Room temperature MilliQ water (18 ± 1 °C) was used in the process. On the basis of preliminary testing, *C. abies-marina* and *Z. tournefortii* were mixed with water during 30 min and 20 min, respectively. Afterwards, the seaweed samples were left to drip for 5 min. An appropriate amount of each seaweed either untreated or subjected to a specific treatment was then freeze-dried, minced, and stored at − 80 °C until analysis.

Proximate composition

The moisture and ash contents were determined according to AOAC methods [15]. Lipid content was determined by the Folch extraction method [16]. The protein level was quantified according to the Dumas method [17] and a conversion factor of nitrogen into protein of 5.0 was used for the seaweed [18]. Carbohydrate content was estimated by difference on the basis of total moisture, ash, and protein contents.

Soluble, insoluble, and total dietary fibre

The fibre content (soluble, insoluble, and total) was determined through an enzymatic procedure as described in a previous work [14]. The contents of insoluble and soluble fibre were corrected by subtracting protein and ash contents in the residues [15, 17]. Analyses were done only for the sun-dried seaweed (duplicate).

Fatty acid profile

Fatty acid methyl esters (FAMEs) were prepared from sun-dried, steam-cooked, and rehydrated seaweed after freeze-drying by acid-catalysed transesterification using the methodology described by Bandarra et al. [19]. The FAMEs were identified by comparing their retention time with those of several Sigma-Aldrich standards (Polyunsaturated Fatty Acids, PUFA-3, Menhaden oil, and PUFA-1, marine source from Supelco Analytical). This analysis was done in triplicate.

Elemental composition

The mineral composition of the initial and bioaccessible samples was determined using a methodology described by Moreira et al. [20]. Prior to analysis, samples underwent an acid digestion that required dry material, thus the bioaccessible samples were placed in an oven for 12 h and dried at 40 ºC. Between 0.3 and 0.5 g of freeze-dried initial samples and dried bioaccessible samples were weighed in duplicate and 7.5 ml of nitric acid (HNO_3) at 65% and 2.5 ml of hydrochloric acid (HCL) at 37% was added. The samples were then submitted to a thermal digestion. After digestion, samples were cooled and diluted to 25 ml with ultra-pure water. The samples were then filtered and kept in labelled tubes. The attained solutions were analysed for Na, Mg, P, S, K, Ca, Fe, Cr, Mn, Cu, Zn, As, Cd, and Pb by Inductively Coupled Plasma Atomic Emission Spectrometry using the equipment Thermo Scientific iCap 7000 (Thermo Fisher Scientific, Inc., Waltham, MA, USA). Analyses were done in duplicate.

Total phenolic content

Phenolic compounds were extracted with water or 96%, v/v, ethanol from the sun-dried, steam-cooked or rehydrated seaweed biomass after freeze-drying [21]—thus matching the extracts used in the assessment of the antioxidant potential (see DPPH method)—and determined by the Singleton and Rossi method [22] using the Folin-Ciocalteu reagent. A volume of 100 µl of each seaweed extract or bioaccessible fraction was used. Gallic acid (GA) was used as standard and phenolic content was expressed as gallic acid equivalents (mg GAE/g dw) through the calibration curve of gallic acid (Sigma, Steinheim, Germany).

Antioxidant activity as measured by the DPPH method

The antioxidant activity was measured through the determination of the radical scavenging activity using 2,2-diphenyl-1-picrylhydrazyl, DPPH [23]. In order to prepare the extracts, approximately 1.25 g of sun-dried, steam-cooked, or rehydrated seaweed biomass after freeze-drying was weighed, homogenized with 25 ml of water or 96%, v/v, ethanol using a model Polytron PT 6100 homogenizer (Kinematica, Luzern, Switzerland) at a velocity of 30,000 rpm during 1 min, and agitated for 18 h on an orbital shaker (400 rpm). After centrifugation (3000×*g* at 4 °C during 10 min), the supernatant was collected through a filter to a final volume of 25 ml. A volume of 1 ml of the extract or the bioaccessibile fraction was used. Either water or 96%, v/v, ethanol was used as the blank and, in the case of the bioaccessible samples, a bioaccessible blank (corresponding to the digestive juices used in the in vitro model) was also analysed for correction. On the basis of an ascorbic acid calibration curve, results were expressed in µg of ascorbic acid equivalents (µg AA Eq) per g dw of the samples.

Antioxidant activity as measured by the FRAP method

The applied Ferric Reducing Antioxidant Power (FRAP) method was a modified technique based on Benzie and Strain [24]. A volume of 100 µl of extract (prepared in 5%, w/v, as described in the DPPH method) or bioaccessible fraction was used. On the basis of a $FeSO_4$ standard curve, linear between 250 and 2000 µM, results were expressed in µmol Fe^{2+} per g dw of the samples.

Antioxidant activity as measured by the ABTS method

ABTS (2,2′-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) radical scavenging activity was determined using the method described by Re et al. [25]. A volume of 20 µl of extract (prepared in 5%, w/v, as described in the DPPH method) or bioaccessible fraction was used. On the basis of a trolox calibration curve, the ABTS radical scavenging activity of the samples was expressed as µmol of trolox equivalents (µmol Trolox Eq) per g dw of the samples.

In vitro digestion model

A static in vitro model replicating human digestion was applied in the determination of bioaccessibility in the

seaweed biomass. The model replicated digestion in three different parts of the GI tract: mouth, stomach, and small intestine. The solutions and enzymes used in this model followed Afonso et al. [9], which was based on Versantvoort et al. [26]. Thus, a non-digested portion and a bioaccessible fraction were attained. Chemicals were supplied by Merck (Darmstadt, Germany) and enzymes by Sigma (St. Louis, MO, USA).

Calculation of bioaccessibility

With exception of elemental bioaccessibility, the percentage (%) of the constituent (C) in the bioaccessible fraction was estimated as follows:

$$\%C\ \text{bioaccessible} = \text{mC bioaccessible} \times 100/\text{mC initial}$$

Being: mC is the mass of constituent.

For elemental bioaccessibility, the percentage (%) of the constituent (C) in the bioaccessible fraction was estimated differently:

$$\%C\ \text{bioaccessible} = \text{mC bioaccessible} \times 100/[S]$$

Being: mC is the mass of constituent; [*S*] is the mC bioaccessible fraction + mC non-digested fraction.

Statistical analysis

Data treatment was done with STATISTICA 10 (Statsof, Inc., USA, 2011). Data were analysed by an one-way ANOVA distribution using the Tukey HSD to determine the difference in the constituents contents/bioactivities between seaweed species or by a factorial ANOVA using the Tukey HSD to determine the difference between seaweed treatments taking into account different extracts and the initial and bioaccessible samples. The significance level (α) was 0.05.

Results

Proximate composition

The proximate composition of the two brown seaweed species *C. abies-marina* and *Z. tournefortii* either untreated or steam-cooked/rehydrated is presented in Table 1.

Firstly, it should be stressed that the sun-dried seaweeds contained still some moisture, in the 6–9%, ww, interval. Of course, steam-cooking and, even more, rehydration increased moisture content. Indeed, while steaming only increased moisture to 17–24%, ww, rehydration expanded moisture to a much higher range, 64–66%, ww. In addition, both sun-dried species had a large share of ash, 25–29%, dw, which did not change much due to steaming. However, rehydration decreased ash content by seven percentage points. Protein level was low in both cases, but significantly higher in *Z. tournefortii*, 11.3–12.2%, dw, *vs* 7.4–8.6%, dw. Protein and lipid contents were much more invariable, being registered a small increase after rehydration. Carbohydrate content increased more with this treatment, from 57–67 to 64–72%, dw, being *C. abies-marina* richer in this component than the other species. Finally, dietary fibre was only determined for the sun-dried seaweeds, showing very similar levels in both species. Insoluble fibre was up to more than 20-fold more important than soluble fibre.

Fatty acid profile

The overall proportion of saturated (SFA), monounsaturated (MUFA), and polyunsaturated (PUFA) almost did not change with treatment, but differed between the two brown

Table 1 Proximate composition (expressed in g/100 g ww or dw) of the *Cystoseira abies-marina* and *Zonaria tournefortii* biomass

Parameter	*C. abies-marina*			*Z. tournefortii*		
	Sun-dried	Steam-cooked	Rehydrated	Sun-dried	Steam-cooked	Rehydrated
Moisture (g/100 g ww)	6.2 ± 0.0	16.7 ± 0.1	66.0 ± 0.1	8.6 ± 0.1	24.4 ± 0.0	64.1 ± 0.0
Ash (g/100 g dw)	$25.2 \pm 0.1^{b\dagger}$	$26.4 \pm 0.1^{c\dagger}$	$17.8 \pm 0.1^{a\dagger}$	$29.3 \pm 0.1^{b\ddagger}$	$31.0 \pm 0.5^{b\ddagger}$	$22.1 \pm 0.4^{a\ddagger}$
Protein (g/100 g dw)	$7.4 \pm 0.2^{a\dagger}$	$7.7 \pm 0.1^{a\dagger}$	$8.6 \pm 0.1^{b\dagger}$	$11.8 \pm 0.2^{ab\ddagger}$	$11.3 \pm 0.2^{a\ddagger}$	$12.2 \pm 0.2^{b\ddagger}$
Fat (g/100 g dw)	$0.8 \pm 0.1^{a\dagger}$	$1.2 \pm 0.1^{ab\dagger}$	$1.3 \pm 0.2^{b\dagger}$	$1.6 \pm 0.2^{a\ddagger}$	$1.2 \pm 0.2^{a\dagger}$	$1.6 \pm 0.1^{a\dagger}$
Carbohydrate (g/100 g dw)	66.6	64.7	72.4	57.3	56.5	64.1
Soluble dietary fibre (g/100 g dw)	1.8	–	–	2.4	–	–
Insoluble dietary fibre (g/100 g dw)	44.4	–	–	43.1	–	–
Total dietary fibre (g/100 g dw)	46.2	–	–	45.5	–	–

Values are presented as the average ± standard deviation. Different lowercase letters within a row correspond to significant differences ($p < 0.05$) between culinary treatments for each seaweed. Different symbols († or ‡) within a row correspond to statistical differences ($p < 0.05$) between seaweed species for the same culinary treatments

seaweed species (Table 2). In fact, for similar MUFA levels in both species, while *C. abies-marina* was richer in PUFA (30–31% vs 20–21%), *Z. tournefortii* was richer in SFA (53–57% vs 46–47%). Likewise, within SFAs, no effect of the treatments was registered on their contents, but myristic acid (14:0) concentration was higher in *Z. tournefortii* than in *C. abies-marina*, 15–17% vs 7–8%. Regarding MUFA, though slight changes were brought about by type of treatment, main FA contents were very similar across treatments and species. Concerning PUFA, owing to the lower ω6 PUFA concentration in *Z. tournefortii*, the relative weight of ω3 PUFA with respect to ω6 PUFA doubled from *C. abies-marina* to *Z. tournefortii*. Main driver in this difference was arachidonic acid (20:4 ω6), present at higher levels in *C. abies-marina*, 14.7–15.4% *vs* 3.8–3.9%. The other main ω6 PUFA, linoleic acid (18:2 ω6), only exhibited minor content fluctuations as a result of treatment or species. The same can be stated regarding stearidonic acid (18:4 ω3). The main contributors to total ω3 PUFA content were different in each species because alpha-linolenic acid (18:3 ω3) was relatively abundant in *C. abies-marina*, 4.5–5.1%, but almost non-existent in *Z. tournefortii*, which had eicosapentaenoic acid (20:5 ω3) as its main ω3 PUFA, 5.8–6.7%.

Elemental composition

Focusing first on the macroelements (Table 3), both seaweed species had high concentrations of Na, S, K, and Ca, all exceeding 10 g/kg dw with exception of K in rehydrated *Z. tournefortii*. The levels of Mg were somewhat lower, in the 4.4–9.9 g/kg dw range, and the levels of P and Fe were always lower than 2 g/kg dw. Species had an effect on K content vs the levels of divalent elements such as Mg and Ca. Indeed, *Z. tournefortii* showed a K concentration that was less than half that determined in *C. abies-marina*. On the other hand, the sum of Mg and Ca concentrations in *Z. tournefortii* was twofold the same sum in the other species. Moreover, while steaming left the macroelement profile largely unaffected in both species, rehydration generated an elemental concentration reduction in most instances. Notable exceptions to this effect were observed in Ca and Fe, whose contents in both species did not decline as a result of rehydration.

With respect to the microelements (Table 4), Mn, Cu, Zn, and As contents were relevant in both species. The levels of Cr and Pb in *C. abies-marina* were below quantification. More specifically, the As content in this species was very high, ranging between 295 ± 5 mg/kg dw and 369 ± 2 mg/kg dw, in rehydrated and steam-cooked seaweed, respectively. It is worth noting that treatment affected As content not only in *C. abies-marina*, but also in *Z. tournefortii*, having rehydration a reducing influence on As levels. No major effect of steaming or rehydration on other miroelemental levels was found. Though *Z. tournefortii* was poorer in As, it was richer in Cr, Mn, Cu, Zn, Cd, and Pb.

Table 2 Fatty acid profile (expressed in % of total fatty acids) of the *Cystoseira abies-marina* and *Zonaria tournefortii* biomass

Fatty acid (% total fatty acids)	*C. abies-marina*			*Z. tournefortii*		
	Sun-dried	Steam-cooked	Rehydrated	Sun-dried	Steam-cooked	Rehydrated
14:0	$6.8 \pm 0.2^{a\dagger}$	$7.6 \pm 0.7^{a\dagger}$	$7.1 \pm 0.5^{a\dagger}$	$16.8 \pm 1.4^{a\ddagger}$	$15.1 \pm 1.8^{a\ddagger}$	$15.6 \pm 0.2^{a\ddagger}$
16:0	$34.3 \pm 1.3^{a\dagger}$	$33.2 \pm 0.5^{a\dagger}$	$34.1 \pm 0.7^{a\dagger}$	$31.5 \pm 2.6^{a\dagger}$	$30.4 \pm 0.9^{a\dagger}$	$28.9 \pm 0.3^{a\dagger}$
Σ SFA	$46.9 \pm 0.2^{a\dagger}$	$45.7 \pm 1.0^{a\dagger}$	$46.4 \pm 0.9^{a\dagger}$	$56.9 \pm 2.8^{a\ddagger}$	$55.1 \pm 2.5^{a\ddagger}$	$53.2 \pm 0.2^{a\ddagger}$
16:1 ω7	$2.2 \pm 0.0^{c\dagger}$	$2.0 \pm 0.0^{b\dagger}$	$1.9 \pm 0.0^{a\dagger}$	$1.4 \pm 0.0^{b\dagger}$	$1.6 \pm 0.0^{c\dagger}$	$1.2 \pm 0.0^{a\dagger}$
18:1 ω9	$18.2 \pm 0.3^{b\dagger}$	$17.5 \pm 0.3^{a\dagger}$	$18.0 \pm 0.2^{ab\dagger}$	$16.0 \pm 1.6^{a\dagger}$	$16.3 \pm 0.9^{a\dagger}$	$15.7 \pm 0.1^{a\dagger}$
Σ MUFA	$21.0 \pm 0.1^{b\dagger}$	$20.2 \pm 0.3^{a\dagger}$	$20.5 \pm 0.2^{ab\dagger}$	$19.9 \pm 0.5^{a\dagger}$	$20.7 \pm 0.9^{a\dagger}$	$19.4 \pm 0.2^{a\dagger}$
18:2 ω6	$5.7 \pm 0.0^{a\dagger}$	$6.3 \pm 0.1^{c\dagger}$	$6.1 \pm 0.1^{b\dagger}$	$6.8 \pm 0.2^{a\ddagger}$	$7.1 \pm 0.5^{a\ddagger}$	$6.8 \pm 0.1^{a\ddagger}$
18:3 ω3	$4.5 \pm 0.0^{a\dagger}$	$5.1 \pm 0.1^{c\dagger}$	$4.9 \pm 0.1^{b\dagger}$	$ND^{a\ddagger}$	$ND^{a\ddagger}$	$0.9 \pm 0.0^{b\ddagger}$
18:4 ω3	$1.2 \pm 0.0^{a\dagger}$	$1.4 \pm 0.1^{b\dagger}$	$1.3 \pm 0.0^{b\dagger}$	$1.0 \pm 0.0^{a\ddagger}$	$1.2 \pm 0.2^{a\dagger}$	$1.2 \pm 0.0^{a\ddagger}$
20:4 ω6	$15.2 \pm 0.5^{a\dagger}$	$15.4 \pm 0.4^{a\dagger}$	$14.7 \pm 0.7^{a\dagger}$	$3.8 \pm 0.1^{a\ddagger}$	$3.9 \pm 0.1^{a\ddagger}$	$3.9 \pm 0.1^{a\ddagger}$
20:5 ω3	$1.9 \pm 0.1^{a\dagger}$	$2.1 \pm 0.1^{b\dagger}$	$1.9 \pm 0.1^{ab\dagger}$	$5.9 \pm 0.4^{a\ddagger}$	$6.7 \pm 0.3^{b\ddagger}$	$5.8 \pm 0.0^{a\ddagger}$
Σ PUFA	$30.2 \pm 0.6^{a\dagger}$	$31.1 \pm 0.3^{b\dagger}$	$30.7 \pm 0.8^{ab\dagger}$	$19.6 \pm 0.5^{a\ddagger}$	$20.0 \pm 1.0^{a\ddagger}$	$20.7 \pm 0.2^{a\ddagger}$
Σ ω3 PUFA	$8.5 \pm 0.2^{a\dagger}$	$9.6 \pm 0.5^{b\dagger}$	$9.1 \pm 0.1^{ab\dagger}$	$8.4 \pm 0.7^{a\dagger}$	$9.3 \pm 0.4^{a\dagger}$	$9.3 \pm 0.0^{a\ddagger}$
Σ ω6 PUFA	$21.4 \pm 0.5^{a\dagger}$	$22.2 \pm 0.5^{a\dagger}$	$21.3 \pm 0.7^{a\dagger}$	$10.9 \pm 0.3^{a\ddagger}$	$11.3 \pm 1.5^{a\ddagger}$	$11.0 \pm 0.2^{a\ddagger}$
Σ ω3/Σ ω6	$0.4 \pm 0.0^{a\dagger}$	$0.4 \pm 0.0^{a\dagger}$	$0.4 \pm 0.0^{a\dagger}$	$0.8 \pm 0.1^{a\ddagger}$	$0.9 \pm 0.2^{a\ddagger}$	$0.8 \pm 0.0^{a\ddagger}$

Values are presented as the average ± standard deviation. Different lowercase letters within a row correspond to significant differences ($p < 0.05$) between culinary treatments for each seaweed. Different symbols († or ‡) within a row correspond to statistical differences ($p < 0.05$) between seaweed species for the same culinary treatments

ND not detected

Table 3 Elemental composition (g/kg dw) and bioaccessibility (%) concerning macroelements in the *Cystoseira abies-marina* and *Zonaria tournefortii* biomass

Element	*C. abies-marina*			*Z. tournefortii*		
	Sun-dried	Steam-cooked	Rehydrated	Sun-dried	Steam-cooked	Rehydrated
[Na] (g/kg dw)	31.5 ± 0.4^{b}	32.5 ± 0.8^{b}	16.0 ± 0.1^{a}	32.7 ± 2.8^{b}	33.2 ± 0.1^{b}	10.2 ± 0.0^{a}
Na Bioaccessibility (%)	18 ± 1^{b}	ND[a]	ND[a]	12 ± 13^{a}	13 ± 12^{a}	2 ± 3^{a}
[Mg] (g/kg dw)	6.0 ± 0.2^{b}	5.9 ± 0.1^{b}	4.4 ± 0.1^{a}	9.4 ± 0.1^{b}	9.9 ± 0.2^{b}	7.4 ± 0.3^{a}
Mg Bioaccessibility (%)	76 ± 0^{b}	74 ± 0^{b}	64 ± 1^{a}	81 ± 0^{a}	78 ± 1^{a}	72 ± 5^{a}
[P] (g/kg dw)	0.35 ± 0.01^{a}	0.40 ± 0.03^{b}	0.26 ± 0.02^{a}	0.61 ± 0.06^{a}	0.65 ± 0.03^{a}	0.53 ± 0.01^{a}
P Bioaccessibility (%)	ND	ND	ND	ND	ND	ND
[S] (g/kg dw)	11.5 ± 0.1^{b}	11.8 ± 0.1^{b}	9.6 ± 0.1^{a}	15.2 ± 0.8^{ab}	16.0 ± 0.8^{b}	12.5 ± 0.6^{a}
S Bioaccessibility (%)	30 ± 1^{b}	31 ± 5^{b}	6 ± 2^{a}	31 ± 2^{b}	30 ± 0^{b}	ND[a]
[K] (g/kg dw)	46.2 ± 1.8^{b}	51.3 ± 0.1^{c}	29.9 ± 0.6^{a}	16.3 ± 1.6^{b}	19.7 ± 0.0^{b}	7.3 ± 0.6^{a}
K Bioaccessibility (%)	72 ± 3^{b}	70 ± 2^{b}	52 ± 1^{a}	64 ± 3^{b}	58 ± 3^{b}	ND[a]
[Ca] (g/kg dw)	11.3 ± 0.4^{ab}	10.7 ± 0.3^{a}	12.2 ± 0.2^{b}	25.8 ± 1.1^{a}	30.7 ± 1.0^{a}	30.1 ± 1.6^{a}
Ca Bioaccessibility (%)	21 ± 9^{a}	17 ± 1^{a}	7 ± 2^{a}	59 ± 2^{a}	61 ± 1^{a}	51 ± 7^{a}
[Fe] (g/kg dw)	0.04 ± 0.00^{a}	0.04 ± 0.00^{a}	0.04 ± 0.00^{a}	1.62 ± 0.13^{a}	1.61 ± 0.13^{a}	1.91 ± 0.34^{a}
Fe Bioaccessibility (%)	ND	ND	ND	ND	ND	ND

Values are presented as the average ± standard deviation. Different lowercase letters within a row correspond to significant differences ($p < 0.05$) between culinary treatments for each seaweed

ND not detected

Table 4 Elemental composition (mg/kg dw) and bioaccessibility (%) concerning microelements in the *Cystoseira abies-marina* and *Zonaria tournefortii* biomass

Element	*C. abies-marina*			*Z. tournefortii*		
	Sun-dried	Steam-cooked	Rehydrated	Sun-dried	Steam-cooked	Rehydrated
[Cr] (mg/kg dw)	< LOQ	< LOQ	< LOQ	5.1 ± 0.6^{a}	5.3 ± 0.5^{a}	4.6 ± 0.6^{a}
Cr Bioaccessibility (%)	ND	ND	ND	ND	ND	ND
[Mn] (mg/kg dw)	2.9 ± 0.1^{a}	3.4 ± 0.3^{a}	3.2 ± 0.3^{a}	36.6 ± 3.7^{a}	37.4 ± 3.6^{a}	40.4 ± 5.0^{a}
Mn Bioaccessibility (%)	ND	ND	ND	10 ± 2^{a}	6 ± 0^{a}	7 ± 9^{a}
[Cu] (mg/kg dw)	6.4 ± 0.2^{a}	5.9 ± 0.2^{a}	5.5 ± 0.9^{a}	8.4 ± 0.3^{a}	8.6 ± 0.0^{a}	8.2 ± 1.2^{a}
Cu Bioaccessibility (%)	ND	18 ± 8^{a}	ND	ND	ND	ND
[Zn] (mg/kg dw)	3.2 ± 0.4^{a}	4.2 ± 0.8^{a}	4.5 ± 1.0^{a}	11.7 ± 1.3^{a}	8.9 ± 0.4^{a}	10.6 ± 1.0^{a}
Zn Bioaccessibility (%)	ND	ND	ND	ND	ND	ND
[As] (mg/kg dw)	340 ± 0^{b}	369 ± 2^{c}	295 ± 5^{a}	57 ± 0^{b}	56 ± 0^{b}	41 ± 3^{a}
As Bioaccessibility (%)	81 ± 1^{b}	73 ± 0^{a}	72 ± 1^{a}	50 ± 0^{b}	50 ± 3^{b}	19 ± 2^{a}
[Cd] (mg/kg dw)	0.27 ± 0.01^{a}	0.28 ± 0.01^{ab}	0.32 ± 0.00^{b}	0.80 ± 0.00^{a}	0.86 ± 0.01^{a}	0.89 ± 0.05^{a}
Cd Bioaccessibility (%)	47 ± 4^{a}	53 ± 6^{a}	35 ± 8^{a}	49 ± 2^{a}	50 ± 2^{a}	46 ± 1^{a}
[Pb] (mg/kg dw)	< LOQ	< LOQ	< LOQ	0.90 ± 0.11^{a}	1.55 ± 0.05^{b}	0.82 ± 0.10^{a}
Pb Bioaccessibility (%)	ND	ND	ND	ND	ND	ND

Values are presented as the average ± standard deviation. Different lowercase letters within a row correspond to significant differences ($p < 0.05$) between culinary treatments for each seaweed

LOQ limit of quantification, *ND* not detected

Phenolic content and antioxidant activity

The highest total phenolic contents in the aqueous extracts were observed in *C. abies-marina*, between 620 and 1280 mg GAE/100 g dw. For *Z. tournefortii*, values were in the 170–280 mg GAE/100 g dw (Table 5). It is worth noting that whereas steaming reduced phenolic content, rehydrating led to the opposite outcome. The extraction with ethanol yielded much lower phenolic contents with exception of rehydrated *Z. tournefortii*. This exception may be related

Table 5 Total phenolic content (in mg GAE/g dw) and antioxidant activity as measured by DPPH (μg AA Eq/g dw), FRAP (μmol Fe^{2+}/g dw), and ABTS (μmol Trolox Eq/g dw) methods in aqueous and ethanolic extracts of the *Cystoseira abies-marina* and *Zonaria tournefortii* biomass and in their bioaccessibile fractions

Parameter	Extract	*C. abies-marina*			*Z. tournefortii*		
		Sun-dried	Steam-cooked	Rehydrated	Sun-dried	Steam-cooked	Rehydrated
Total phenolic content (mg GAE/g dw)	Aqueous	$8.4 \pm 0.6^{bB\dagger}$	$6.2 \pm 0.2^{aB\dagger}$	$12.8 \pm 0.9^{cC\dagger}$	$2.4 \pm 0.0^{bB\ddagger}$	$1.7 \pm 0.0^{aC\ddagger}$	$2.8 \pm 0.0^{cB\ddagger}$
	Ethanolic	$0.8 \pm 0.1^{aA\dagger}$	$1.0 \pm 0.0^{abA\dagger}$	$1.5 \pm 0.3^{bA\dagger}$	$1.2 \pm 0.1^{abA\ddagger}$	$1.1 \pm 0.0^{aA\ddagger}$	$2.6 \pm 0.2^{bB\ddagger}$
	Bioacc	$8.9 \pm 1.1^{cB\dagger}$	$7.2 \pm 0.3^{bC\dagger}$	$3.8 \pm 0.5^{aB\dagger}$	$2.1 \pm 0.5^{cB\ddagger}$	$1.4 \pm 0.1^{bB\ddagger}$	$0.8 \pm 0.2^{aA\ddagger}$
DPPH (μg AA Eq/g dw)	Aqueous	$501 \pm 13^{cC\dagger}$	$453 \pm 1^{bB\dagger}$	$434 \pm 2^{aB\dagger}$	$418 \pm 14^{cB\ddagger}$	$369 \pm 5^{bC\ddagger}$	$174 \pm 4^{aB\ddagger}$
	Ethanolic	$456 \pm 2^{aB\dagger}$	$475 \pm 4^{bC\dagger}$	$472 \pm 2^{bC\dagger}$	$434 \pm 5^{bB\ddagger}$	$350 \pm 2^{aB\ddagger}$	$360 \pm 3^{aC\ddagger}$
	Bioacc	$19 \pm 8^{abA\dagger}$	$29 \pm 8^{bA\dagger}$	$9 \pm 5^{aA\dagger}$	$128 \pm 19^{aA\ddagger}$	$119 \pm 5^{aA\ddagger}$	$127 \pm 22^{aA\ddagger}$
FRAP (μmol Fe^{2+}/g dw)	Aqueous	$113 \pm 8^{bC\dagger}$	$65 \pm 4^{aA\dagger}$	$107 \pm 3^{bC\dagger}$	$25 \pm 0^{aB\ddagger}$	$28 \pm 1^{bC\ddagger}$	$33 \pm 0^{cC\ddagger}$
	Ethanolic	$52 \pm 4^{aA\dagger}$	$105 \pm 1^{cB\dagger}$	$72 \pm 4^{bB\dagger}$	$34 \pm 4^{bC\ddagger}$	$23 \pm 1^{aB\ddagger}$	$23 \pm 4^{aB\ddagger}$
	Bioacc	$79 \pm 9^{cB\dagger}$	$62 \pm 4^{bA\dagger}$	$43 \pm 5^{aA\dagger}$	$9 \pm 2^{bA\ddagger}$	$2 \pm 1^{aA\ddagger}$	$3 \pm 1^{aA\ddagger}$
ABTS (μmol Trolox Eq/g dw)	Aqueous	$43 \pm 1^{aB\dagger}$	$144 \pm 36^{bB\dagger}$	$212 \pm 1^{cB\dagger}$	$35 \pm 1^{aA\ddagger}$	$50 \pm 3^{bB\ddagger}$	$62 \pm 4^{cB\ddagger}$
	Ethanolic	$16 \pm 1^{aA\dagger}$	$20 \pm 1^{bA\dagger}$	$22 \pm 1^{bA\dagger}$	$37 \pm 1^{cA\ddagger}$	$25 \pm 1^{bA\ddagger}$	$24 \pm 0^{aA\dagger}$

Values are presented as the average ± standard deviation. Different lowercase letters within a row correspond to significant differences ($p < 0.05$) between culinary treatments for each seaweed. Different uppercase letters within a column correspond to significant differences ($p < 0.05$) between extracts (aqueous, ethanolic, bioaccessible fraction) for each culinary treatment. Different symbols († or ‡) within a row correspond to statistical differences ($p < 0.05$) between seaweed species for the same culinary treatments

ND not detected

to an enhancement of the phenolic content in the ethanolic extracts of the rehydrated seaweed that parallels the trend in the aqueous extracts. Differently from the aqueous extracts, the *Z. tournefortii* ethanolic extracts were richer in phenolic compounds than the *C. abies-marina* ethanolic extracts.

The FRAP and ABTS methodologies applied to the aqueous extracts yielded results with strong parallelisms to the phenolic contents (Table 5). In particular, the antioxidant activity of the aqueous extracts of *C. abies-marina* as measured by these techniques was higher than that of the same extracts of *Z. tournefortii*. With exception of FRAP in rehydrated *C. abies-marina*, it was observed an increase of the antioxidant activity (FRAP and ABTS) with rehydration. For instance, the ABTS of the aqueous extracts increased from 43 ± 1 to 212 ± 1 μmol Trolox Eq/g dw and from 35 ± 1 to 62 ± 4 μmol Trolox Eq/g dw in *C. abies-marina* and *Z. tournefortii*, respectively. Excluding sun-dried *Z. tournefortii*, ethanolic extracts displayed lower antioxidant activities determined by FRAP and ABTS than aqueous extracts. Rehydration of *Z. tournefortii* did not produce an enhancement of antioxidant activity (FRAP and ABTS) in the ethanolic extracts.

The DPPH method provided a distinct evaluation of the antioxidant activity (Table 5). There was no clear unidirectional change from the aqueous to the ethanolic extracts. For both extract types, DPPH values were lower in *Z. tournefortii* than in *C. abies-marina*. With the sole exception of the ethanolic extracts of *C. abies-marina*, rehydration had a depressing effect on the antioxidant activity. This effect was stronger in the aqueous extracts of *Z. tournefortii* with DPPH declining from 418 ± 14 μg AA Eq/g dw in sun-dried samples to 174 ± 4 μg AA Eq/g dw in rehydrated ones.

Bioaccessibility

The bioaccessibility (%) of the macroelements (Na, Mg, P, S, K, Ca, and Fe) and microelements (Cr, Mn, Cu, Zn, As, Cd, and Pb) present in the biomass of both seaweed species (*C. abies-marina* and *Z. tournefortii*) as subjected to the alternative treatments (sun-dried, steamed, and rehydrated) is presented in Tables 3 and 4, respectively. On the other hand, the bioaccessible levels of the phenolic compounds and antioxidant activity (DPPH and FRAP) are displayed in Table 5.

Regarding macroelements, Na, P, S, and Fe bioaccessibility was low, not exceeding 31%. In the case of Ca, low bioaccessibility, ranging between 7 and 21%, was calculated for *C. abies-marina*, but 51–61% bioaccessibility was determined for *Z. tournefortii*. The opposite was found for K bioaccessibility, since it varied between 52 and 72% in *C. abies-marina* and decreased from 64 ± 3% in sun-dried *Z. tournefortii* to non-bioaccessible in rehydrated *Z. tournefortii*. Finally, Mg bioaccessibility was generally high across the different seaweed samples, being 64–76% in the variously treated *C. abies-marina* and 72–81% in the equivalent samples of *Z. tournefortii*. In the cases of S and K, rehydration led to lower bioaccessibility for both seaweed species.

Concerning microelements, only As and Cd showed relatively high bioaccessibility levels in the various seaweed samples. Namely, As bioaccessibility ranged between 72 and

81% in *C. abies-marina* and varied at a lower level (19–50%) in *Z. tournefortii*. On the other hand, Cd bioaccessibility varied in similar ranges for both seaweed species, 35–53% for *C. abies-marina* and 46–50% for *Z. tournefortii*. In the case of Cr, Mn, Cu, Zn, and Pb, bioaccessibility was always below 20% and frequently the elemental content was undetected in the bioaccessible fraction. Rehydration had a lowering action upon the As bioaccessibility, reducing it from 81 ± 1% (sun-dried) to 72 ± 1% for *C. abies-marina* and from 50 ± 0% (sun-dried) to 19 ± 2% for *Z. tournefortii*. However, no similar action was registered for the other elements.

Rehydration had also a reducing effect on the bioaccessibility of the phenolic compounds as shown by the significantly lower bioaccessibile phenolic contents with respect to the initial (prior to digestion) contents as extracted with water. For all treatments and species, antioxidant bioaccessibility as measured by DPPH was almost always lower than 50%, being even lower than 10% in all *C. abies-marina* samples. In the case of FRAP, while *Z. tournefortii* samples presented bioaccessibility values below 40%, *C. abies-marina* samples' values were always above 40%. For both seaweed species, rehydration depressed FRAP bioaccessibility in comparison to the sun-dried samples.

Discussion

Proximate composition

Both applied treatments (steam-cooking and rehydration) led to a higher moisture content, but none of them, not even rehydration, enabled to fully recover the typical moisture content of fresh brown seaweeds, near 80% ww [27]. The large increase of moisture with rehydration helps explain the observed reduction of ash content on a dry matter basis. The mineral fraction is largely composed of salts that were dissolved and extracted more thoroughly with a deeper and more intense contact of the seaweeds with water. Regarding protein, its low levels agree with the literature [5, 27, 28]. Namely, low protein contents in the *Cystoseira* genus (*C. barbata*) have been reported by Manev et al. [29] with a 5–13%, dw, range, which agrees with protein content determined in *C. abies-marina*. For *Z. tournefortii*, Nunes et al. [5] reported 9.4 ± 0.1%, dw, only slightly lower than in the current study. Furthermore, other brown seaweeds have been reported to contain between 8 and 13%, dw, protein [28]. The absence of large variations in the protein (and lipid) content of the treated seaweed may result from its structural role and poor solubility in water. These reasons may also explain why the carbohydrate content did not decrease with either steaming or rehydration, especially if it is taken into account that dietary fibre (a major component of the carbohydrate fraction) is overwhelmingly composed by insoluble components (Table 1). An increase of the carbohydrate content after rehydration must be viewed as an indirect consequence of the loss of mineral components that brought about a relative enrichment of the other components. It is also worth noting that a comparison with the literature shows similar total fibre levels in *Z. tournefortii* from the Madeira Archipelago [5].

Fatty acid profile

Given the low lipid contents in seaweed, there are very few studies on this subject. According to literature [30], seaweeds from the *Cystoseira* genus, such as *C. hakodatensis*, are relatively rich in PUFA, up to 50% of total FAs. On the other hand, *Z. tournefortii* has been found to be a much poorer source of PUFA, not exceeding 20% [31]. This generally agrees with the current study. However, the contents of arachidonic and eicosapentaenoic acids did not exceed 3% of total FAs in *Z. tournefortii* from the Madeira Archipelago [31], which are clearly below the concentrations in current study. The higher arachidonic contents in *C. abies-marina* are corroborated by the study of Airanthi et al. [30] on *C. hakodatensis*, which determined 14.6% of the total FAs for this ω6 PUFA, a value remarkably similar to those measured in this study. In addition, the levels of linoleic acid and alpha-linolenic acid were slightly higher in *C. hakodatensis* [30] than in *C. abies-marina*. On the other hand, for the ω3 PUFAs stearidonic and eicosapentaenoic acids, their concentrations in *C. hakodatensis* [30] were multiples of the percentages observed in *C. abies-marina*. In general, any discrepancies may well relate either to the particular species or to the harvest season [32]. Both *C. abies-marina* and *C. hakodatensis* were harvested in June, but in distinct geographical areas. Likewise, the Madeira Archipelago has abiotic conditions distinct from those in the Azores Archipelago, thus explaining any divergence between *Z. tournefortii* in this study and in the Nunes et al. study [31]. It is also worth remarking that a study on *C. indica* showed an eicosapentaenoic acid content of 2.9 ± 0.1% of total FAs (June) that almost matches the content registered in the current study. Moreover, regarding the main MUFA, 16:1 ω7 and oleic acid (18:1 ω9) contents in *C. indica* in June were quite similar to those determined for *C. abies-marina*. The same is valid in the *C. indica* vs *C. abies-marina* comparison for the main SFA, myristic and palmitic (16:0) acids.

Finally, the absence of large variations as a result of the application of particular treatments is expected, given the low extractability of lipophilic components by water. Moreover, the moderate temperatures and short times of steaming would not be enough to cause significant degradation of the PUFA [33].

Elemental composition

Concerning elemental composition, results found for *C. abies-marina* agree with the available literature, especially regarding the high K concentrations in seaweeds of the genus *Cystoseira* [34]. More specifically, these authors studied five species of this genus (with exception of *C. abies-marina*) and determined contents ranging between 16 and 60 g/kg dw, 9 and 27 g/kg dw, and 6 and 19 g/kg dw, for K, Ca, and Mg, respectively. These intervals encompass the values determined for these elements in the sun-dried *C. abies-marina* and, to a great extent, in the steamed and rehydrated seaweed. However, the Na contents in the five *Cystoseira* species were lower than in sun-dried *C. abies-marina*, not surpassing 16 g/kg dw [34]. This divergence may be ascribed to *C. abies-marina* being washed with seawater and sun-dried, while the other *Cystoseira* species were washed with freshwater [34]. Contrastingly, Fe and Zn contents in these five species were higher than in the current study. For instance, *C. baccata*, which had the lowest Fe level among studied seaweeds, had 110 mg/kg dw, and *C. compressa*, the poorest seaweed in Zn, had 9.4 mg/kg dw [34].

The high As level in *C. abies-marina* deserves a special attention. In the available literature, presented values are much lower. For instance, *C. barbata* and *Cystoseira* spp. from the Mediterranean Sea have As levels of 4–6 mg/kg dw, and 20 ± 1 mg/kg dw, respectively [29, 35]. However, among the studied seaweed species, this *Cystoseira* spp. had the highest level of As accumulation [35]. In a highly polluted area, such as the Venice lagoon, *C. barbata* had the highest As concentration among studied species, 242 ± 104 mg/kg dw (maximum of 360 mg/kg dw) [36]. It has been claimed that *C. barbata* is an As hyperaccumulating species (As contents exceeding 100 mg /kg dw) [37]. This may also apply to *C. abies-marina*. Taking into account that this species was harvested in a Mid-Atlantic area that is not heavily polluted, further research, including As speciation, is warranted.

Regarding *Z. tournefortii* and the *Zonaria* genus, there is even less available data than for *C. abies-marina* and the *Cystoseira* genus. Nonetheless, a study on *Z. subarticulata* [38] reported high As levels, 91–92 mg/kg dw, not very different from the current study's levels. These are high values, but much lower than in *C. abies-marina*. The Pb levels in *Z. subarticulata* were also in the same range observed in *Z. tournefortii* [38]. *Z. tournefortii* also has relatively high levels of Mg, Ca, Mn, Fe, and Zn in comparison to the other studied Azorean seaweed. In the case of Fe, its content largely exceeds those concentrations determined in the genera *Fucus*, *Laminaria*, *Undaria*, *Chondrus*, and *Porphyra*, whose Fe content is within the 33–103 mg/kg dw [39]. On the other hand, its Zn content is clearly below the high range measured for these commercial seaweeds, 17–71 mg/kg dw [39]. It is also worth noting that the high content of Ca and other elements with divalent cations may be related to the possible presence of high levels of alginate (or other anionic polysaccharides) in *Z. tournefortii*, given the chemical affinity of alginate toward divalent metals [40].

Steam-cooking did not generate relevant changes in the elemental composition of both seaweed species, thus matching the absence of a reduction of ash content in the steamed samples (Table 1). On the other hand, rehydration involved a much more extensive phenomenon of water absorption and a concomitant steep reduction of the ash content. Accordingly, most macroelements exhibited concentration reductions due to rehydration. This was particularly evident in elements with monovalent cations, such as Na and K, with 30% and larger declines. The concentrations of elements with divalent cations, such as Ca and Fe, did not shown any reduction by rehydration. Likewise, for microelements, rehydration did not cause any concentration decrease with exception of As. Though this makes As-rich seaweed rehydrating an advisable procedure, it should be remarked that reductions did not exceed 30%. This loss could be mainly related to the share of inorganic As, As^{3+} and As^{5+}, in the studied seaweed species [41]. The other microelements have typically relevant shares of divalent cations, such as Cr^{2+}, Mn^{2+}, Cu^{2+}, Zn^{2+}, Cd^{2+} or Pb^{2+}, and the presence of alginate [40] or other anionic polysaccharides with affinity for divalent cations may hamper their removal by water.

Phenolic content and antioxidant activity

Regarding aqueous extracts, the phenolic content in *C. abies-marina* was clearly above the interval mentioned in the literature for seaweed rich in polyphenols, 100–500 mg GAE/100 g dw [42]. This seems to correspond to a general phenomenon in several species from the genus *Cystoseira*, whose aqueous extracts may even exceed the current study's results [43]. On the other hand, a study on *C. indica* has reported a phenolic content range between 80 and 130 mg GAE/100 g dw [32] and another study on *C. hakodatensis* [44] has found a phenolic level of only 9 mg GAE/100 g dw. Besides these results from aqueous extracts, approximately 20 mg GAE per 100 g of ethanolic extract was determined for *C. osmundacea* [45]. With respect to *Z. tournefortii*'s aqueous extracts, their phenolic contents, albeit not very different, are higher than other values reported in the literature, for instance, in Mediterranean *Z. tournefortii*, 78 mg GAE/100 g dw [46]. However, Nunes et al. [5] determined a total phenolic content surpassing 2000 mg GAE/100 g dw for *Z. tournefortii* from the Madeira Archipelago. Such wide range of values may be associated to environmental UV radiation level and its variation with location and season, since higher phenolic contents in seaweed have been ascribed to a more intense UV radiation [47]. The increase of the phenolic content with rehydration may correspond

to a relative enrichment in phenolic compounds as other hydrophilic seaweed components were washed away (e.g. Na, K, etc.). Though phenolic compounds are extractable with water—as clearly seen in the high phenolic contents of the aqueous extracts of *C. abies-marina*—, rehydration treatment cannot be equated to an extraction, since aqueous extractions were done with intense homogenization and lengthy agitation.

Antioxidant activity regardless of used methodology (DPPH, FRAP, and ABTS) was higher in the aqueous extracts of *C. abies-marina* than in the same extracts of *Z. tournefortii*, thus being similar to the phenolic content results and proving the importance of the phenolic compounds as antioxidant substances. The *C. abies-marina*'s and *Z. tournefortii*'s results also compare favourably with other studies on other brown seaweed species, such as *Saccharina japonica*, ABTS activity of approximately 20 µmol Trolox Eq/g dw [48], or *H. scoparia* and *P. binghamiae*, 50–60 µmol Trolox Eq/g dw [49]. Concerning seaweed treatments and aqueous extracts, rehydration led in most cases to an enhancement of antioxidant activity measured by FRAP and ABTS—related to the enrichment in phenolic compounds previously mentioned—, but to a reduction of DPPH values. This latter variation may be ascribed to the loss of highly hydrophilic antioxidant compounds, whose identity requires further research.

Bioaccessibility

Low bioaccessibility has been observed for various elements in other studies on seaweed [10, 50, 51]. This observation may result from the inability of the human digestive enzymes in breaking down the polysaccharides that constitute the cell walls of algal cells [52]. Namely, humans lack the ability to digest β (1 → 4) linkages in glucan polysaccharides, as in cellulose and hemicelluloses such as xyloglucan, but also haven't any alginase. Therefore, those micro- and macroelements with higher bioaccessibility contrast with previous studies. This is the paradoxical case of Ca in *Z. tournefortii*, given the possible association of Ca^{2+} to alginate. This may also be the case of Mg in both seaweed species. Hence, there is some other unexplained phenomenon warranting further research that causes a bioaccessibility higher than 50% in these instances. Moreover, the high As bioaccessibility in *C. abies-marina*—the species containing the highest As contents prior to digestion— is a serious concern, contrasting with previous studies on As bioaccessibility in *Ulva rigida* [50] or *Enteromorpha* sp. [51]. Whereas, in the former case, As bioaccessibility was 17 ± 2%, in the latter case, As bioaccessibility did not surpass 32 ± 2%. However, As bioaccessibility exceeded 90% in *Rhizoclonium riparium* and 70% in *Fucus* sp., a brown seaweed [53]. The high As bioaccessibility may depend on the specific As species in each seaweed species. Indeed, a high percentage of As in seaweed may be found in the form of arsenosugars [54], whose water solubility is high. However, since rehydration was not efficient in removing most As, more research on this subject is needed.

The high bioaccessibility of phenolic compounds in sun-dried and steam-cooked seaweed may be explained by their hydrophilicity. However, phenolic bioaccessibility was low in rehydrated seaweed and some phenolic substances, such as ferulic acid, have been reported to be almost non-bioaccessible [55]. It can be hypothesized that the rehydration led the phenolic compounds to establish different linkages or associations with the other seaweed components, thereby attaining a stronger attachment to the matrix. For both seaweed species, the low DPPH bioaccessibility opposes the high phenolic bioaccessibility and reinforces the hypothesis that compounds other than polyphenols may be responsible for this antioxidant activity. The FRAP methodology yielded results that differed both from those of the phenolic content and DPPH. However, the FRAP bioaccessibility reduction with rehydration may partially reflect the phenomenon observed with the phenolic compounds. Taken together, DPPH and FRAP results indicate that only a small share of the original antioxidant potential in these studied seaweed species is rendered bioaccessible. A recent study on bioaccessible antioxidant potential, albeit on fruit, showed that the antioxidant capacity, determined by DPPH and FRAP, after in vitro digestion decreased 51–78% when compared to the crude extract [56], thus corroborating the current study's conclusion that bioaccessibility is a very meaningful factor to be taken into account in the assessment of any possible health benefits derived from a high antioxidant potential in the original biological material.

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Compliance with ethical standards

Conflict of interest There is no conflict of interest involving any of the authors.

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