Research Paper

Functional and nutritional value of the Chilean seaweeds *Codium fragile, Gracilaria chilensis* and *Macrocystis pyrifera*

Jaime Ortiz[1], Edgar Uquiche[2], Paz Robert[1], Nalda Romero[1], Vilma Quitral[1] and Catherine Llantén[1]

[1] Department of Food Science and Chemical Technology, Faculty of Chemical and Pharmaceutical Science, Universidad de Chile, Santiago, Chile

[2] Department of Chemical Engineering, Universidad de La Frontera, Temuco, Chile

The nutritional composition of the edible seaweeds *Codium fragile*, *Gracilaria chilensis* and *Macrocystis pyrifera* was determined, including amino acid and fatty acid contents along with tocol and carotenoid contents. The results indicated that the three algae *C. fragile*, *G. chilensis* and *M. pyrifera* showed a high range of protein contents (13.7–10.8%), amino acid contents (1879.6–1417.7 mg/100 g dry algae) and a low content in lipids (0.7–15.0%). The most abundant fatty acids in *C. fragile* and *G. chilensis* were linolenic, oleic, and linoleic acid. δ-Tocopherol and α-tocopherol (677.8 and 453.5 μg/g lipid, respectively) were found in *C. fragile*, while in *G. chilensis* and *M. pyrifera,* γ-tocotrienol and α-tocopherol (263.5 and 1327.7 μg/g lipid, respectively) were found. In addition, in *C. fragile* and *G. chilensis* β-carotene was the principal carotenoid found (197.9 and 113.7 μg/g dry algae, respectively) compared with *M. pyrifera* (17.4 μg/g dry algae). The composition of macronutrients (minerals, carbohydrate-type dietetic fiber, proteins; low in lipids) and micronutrients (essential amino acids, PUFA of balanced *n*-6/*n*-3 proportion, β-carotene and α-tocopherol as source of vitamins A and E), corroborate the nutritional and biological potential of the studied algae, which constitute useful raw materials for the development of diets or ingredients for human and animal nutrition.

Keywords: Amino acids / Carotenoids / Fatty acids / Seaweeds / Tocopherols

Received: June 4, 2008; accepted: October 3, 2008

DOI 10.1002/ejlt.200800140

1 Introduction

In Chile there are about 550 species of seaweeds, which grow in benthic habitats, but only about 1% of them are widely known. The most common species are exported as raw material to produce ■ pls check■ carrageenans, alginate and agar-agar. To a lesser degr[illegible]ey are directly ingested as food [1, 2]. But in recent years, the economic and nutritional importance of this renewable natural resource has significantly increased.

Although seaweeds are not a main source of energy, they have been reported to have nutritional value by their vitamin, protein and mineral contents [3, 4]. It is said that 100 g of seaweed provides more than the daily requirement of vitamins A, B_2 and B_{12} and two thirds of the vitamin C requirements [2], and it has been determined that seaweeds are an important source of dietary fiber, mainly soluble fiber [5], which is considered important in preventing constipation, colon cancer, cardiovascular disease and obesity, among others [6–8]. Another distinctive property of sea plants is that they are considered natural sources of hydrosoluble and liposoluble vitamins, such as thiamine and riboflavin, β-carotene and tocopherols, egg ■ egg?■, as well as of long-chain polyunsaturated essential fatty[illegible]s from the *n*-3 family (*n*-3 LC-PUFA), such as eicosapentaenoic acid (EPA, 20:5*n*-3) [9–11], which may reduce the risk of heart disease, thrombosis and atherosclerosis. The antiviral activity of the fatty acids of certain seaweeds has also been reported [12]. The red alga *Gracilaria chilensis*, belonging to the Gracilariaceae family and classified as a Rhodophycea, is known as "pelillo" on account of its appearance. It has a long and filamentous thallus. It is a reddish brown alga, with variable branching reaching 2 m. It grows in bunches or isolated, in habitats with solid substrates [13]. This alga is almost entirely used in the domestic and

Correspondence: Jaime Ortiz, Department of Food Science and Chemical Technology, Faculty of Chemical and Pharmaceutical Science, Universidad de Chile, P.O. Box 233-D, Santiago, Chile.
E-mail: jaortiz@uchile.cl
Fax: + 56 2 2227900

foreign industry for the development of agar, and is one of the most exported (126,000 t/year) [14]. *Macrocystis pyrifera*, belonging to the Lessoniaceae family and classified as a Phaeophycea, is known a "huiro" and "kelp", reaching a length of 30 m and adhering to a substrate through a base conical disc [15]. In general, *M. pyrifera* is produced for national internal consumption and for exportation with a price of US$600/t. This alga serves as a substrate to a significant number of shellfish, sea urchins and fish. In addition, due to its large size and mobility, it helps in the oxygenation of the sea. It has a high content of iodine and sodium, which are probably not sufficiently consumed by people suffering from hyperthyroidism and hypertension, respectively. Thus, to check its nutritional value can contribute to the development of new sources of diets for human consumption as well as for aquiculture with emphasis on the farming of fish of high added value, such as salmon [16]. The green alga *Codium fragile* ssp. *tomentosoides* (Chlorophyta) is a recognized invader in marine ecosystems around the world, with described ecological effects ranging from minor changes in native species abundance to major changes in community structure, as well as negative economic effects on aquaculture species, especially for *G. chilensis* farming [17]. Thus, it would be important to control its proliferation by means of the industrial exploitation of this raw material or of its derivatives. There is interest in the use of edible seaweeds for the development of low-cost, highly nutritive diets for human and animal nutrition, especially the latter since sea vegetables are able to accelerate the growth of species such as big oysters, tilapia and trout, all of which are of great commercial interest [18, 19].

The aim of the present investigation was to study the nutritional value and some bioactive compounds (tocopherols, tocotrienol and carotenoids) of the *G. chilensis*, *M. pyrifera* and *C. fragile* species, which represent natural resources with potential economic value for use in human and animal nutrition.

2 Materials and methods

2.1 Sample collection

The three algae were collected in January 2007, *G. chilensis* and *C. fragile* were collected raw and fresh from the coastal area of Northern Chile and *M. pyrifera* was supplied dried and milled (flour) by the Marine Cultivation Company Caldera (Caldera, Chile).

2.2 Proximal analysis

Following official AOAC [20] methods, water content (AOAC 934.01), ash (AOAC 930.05), and proteins (N 6.25, AOAC 954.01) were determined. Carbohydrate contents were obtained by difference of 100% – (%protein + %lipid + %ash) [20].

2.3 Lipid extraction

Lipids were extracted using a modification of the Folch method according to Christie [21]. Each homogenized seaweed was extracted with 15 mL chloroform/methanol/water (1 : 2 : 0.8, vol/vol/vol) overnight in the absence of light. Three extractions were performed with ultrasonication and centrifugation. The extracts from each sample were partitioned against chloroform/water (1 : 1, vol/vol) considering the water sample content to give a final chloroform/methanol/water ratio of 1 : 1 : 0.9 (vol/vol/vol). NaCl (5%) was added to the aqueous phase in order to help the separation. For each sample, the organic phases were combined and concentrated in a Büchi RE120 (Büchi Labortechnik, Switzerland) evaporator at 40 °C. Total lipids were gravimetrically determined on aliquots of each lipid extract.

2.4 Amino acid analysis

Amino acids were determined by high-performance liquid chromatography (HPLC) by the method of Alaiz *et al.* [22]. Algae were ground with mortar and pestle. A sample equivalent to 2 mg of protein was weighed into a hydrolysis tube and then 4 mL 6.0 M hydrochloric acid was added. D,L-α-Aminobutyric acid was used as internal standard. The solution was gassed with nitrogen and sealed and then it was incubated in an oven at 110 °C for 24 h. The amino acid hydrolyzate was dried in a Büchi Rotavapor RE120 (Büchi Labortechnik) and the amino acids were dissolved in 25 mL borate buffer (1 M, pH 9.0). Of this solution, 5 mL was derivatized with 4 μL diethyl ethoxymethylene malonate at 50 °C for 50 min with vigorous shaking. Of this derivatized solution, 20 μL was injected directly into the HPLC system. The HPLC system consisted of a Merck-Hitachi L-6200A pump (Merck, Darmstadt, Germany), a Rheodyne 7725i injector with a 20-μL sample loop, and a Merck-Hitachi D-2500 chromato-integrator. The separation of derivatives was attained using a 300 × 3.9 mm i.d. reversed-phase column (Nova-Pack C_{18}, particle size 4 μm; Waters, Milford, MA, USA). Detection was accomplished using a Model L-4250 UV-VIS detector (Merck-Hitachi) with the variable-wavelength monitor set at 280 nm. Resolution of amino acid derivatives was routinely accomplished using a binary gradient system. The solvents used were (A) 25 mM sodium acetate containing 0.02% (vol/vol) sodium azide (pH 6.0) and (B) acetonitrile. Solvent was delivered to the column at a flow rate of 0.9 mL/min as follows: 0.0–3.0 min, linear gradient from A/B (92 : 8) to A/B (88 : 12); 3.0–6.0 min, linear gradient from A/B (88 : 12) to A/B (86 : 14); 6.0–13.0 min, elution with A/B (86 : 14); 13.0–22.0 min, linear gradient from A/B (86 : 14) to A/B (79 : 21); 22.0–35.0 min, linear gradient from A/B (79 : 21) to A/B (69 : 31).

VCH Auftr.-Nr. 36106 Vers.: 6.06
S:/3d/vch/_EJLST/ej_09_03/fl_0140.3d
EJLST
1. Papierl. Seite 2 – SW –
Donnerstag, den 18. 12. 08, 13 Uhr 05

2.5 Fatty acid composition

The fatty acid composition was determined by GLC using an HP 5890 FID detector (Hewlett-Packard, Palo Alto, CA, USA) and a 50-m fused-silica BPX70 capillary column 0.25 µm film, temperature programmed between 160 and 230 °C, rate 2 °C/min, with hydrogen as carrier and using a fatty acid methyl ester (FAME) reference from Merck for identification. FAME were prepared according to AENOR [23].

2.6 Tocopherol and tocotrienol analysis

Tocopherols and tocotrienols were determined in the lipid extracts by HPLC with fluorescence detection, following the AOCS standard method Ce 8-89 [24]. A LichroCART Superspher Si 60 column (25 cm × 4 mm i.d., particle size 5 µm; Merck) was used. The mobile phase was propan-2-ol in hexane (0.5 : 99.5, vol/vol) at a flow rate of 1 mL/min. The HPLC system consisted of a Merck-Hitachi L-6200A pump (Merck), a Rheodyne 7725i injector with 20-µL sample loop, a Merck-Hitachi F-1050 fluorescence detector and a Merck-Hitachi D-2500 chromato-integrator. Peaks were detected at 290 and 330 nm excitation and emission wavelengths, respectively. Tocols were identified using external standards (Merck).

2.7 Carotenoid pigments

Dry algae (5 g) were reconstituted by adding water and then ground in a mortar with cold acetone and 5 g celite (Merck). Three extractions were made until the residue had no color. The combined acetone extracts were transferred into petroleum ether [25] and then saponified with an equal volume of 10% potassium hydroxide in methanol, overnight at room temperature. The mixture was placed in a separatory funnel. The carotenoid solution was washed with distilled water to remove the alkali and then filtered through anhydrous sodium sulfate. Finally, the solvent was evaporated in a rotavapor, obtaining a final volume of 25 mL.

Carotenoid analysis was carried out by HPLC using a Symmetry RP-18 column (5 µm particle size, 4.6 mm ■ pls check■ i.d. × 25 cm; Waters). A mobile phase of methanol/acetonitrile/ethyl acetate (20 : 65 : 15, vol/vol/vol) was used at a flow rate of 1 mL/min. The HPLC system consisted of a Merck-Hitachi L-6200A pump and a Waters diode array 9962 detector. External standards of carotenoid pigments were used (Roche, USA).

2.8 Expression of data and statistical analysis

All data presented are means ± standard deviation ($n = 6$).

3 Results and discussion

3.1 Protein and ash

The nutritional composition of the seaweeds under study, expressed as 100 g dry weight, is shown in Table 1. The protein contribution of *C. fragile*, *G. chilensis* and *M. pyrifera* ranged from 10.8 to 13.7 g/100 g dry weight. In the case of *M. pyrifera*, which is a Phaeophycea, the amount of protein was similar to that of *G. chilensis*, with values of 13.2 and 13.7 g/100 g dry weight, respectively. As reference it may be said that the protein contribution of the three algae is similar to that of some terrestrial plants, vegetables, seeds, grains, eggs, among others, pointed out by several authors [26–28]. The contribution of biologically good proteins makes these algae suitable for inclusion both in animal (especially marine species) and in human food [27, 29–31].

The ash content obtained from the three algae ranged from 10.8 to 20.9 g/100 g dry food. The highest concentration is shown for *C. fragile*, as indicated in Table 1, with all values being higher than those reported for several vegetables of usual consumption such as Swiss chard, spinach and some algae [27, 29, 32–34]. The high amount of organic matter of these species should be pointed out, *i.e.* mainly the high content of minerals such as sodium, iron, sulfur, iodine, calcium, magnesium, phosphorus and potassium, some of which are essential for the proper functioning of the body [35, 36], due to the great capacity of these algae to store these minerals from the marine environment where they live [3, 37].

3.2 Lipids

The lipid content was very low for the three algae under study, ranging from 0.7 to 1.5 g/100 g dry weight, which are the limit values for *M. pyrifera* and *C. fragile*, respectively. In the case of *G. chilensis*, its fat contribution reached 1.3 g/100 g. The overall fat contribution of these three algae is similar to that of cereals (rice and rye) and legumes (common beans, chick-pea and broad bean), which is less than 2%, and slightly above that of most usually consumed terrestrial plants, ranging from 0.2 to 0.8% fat as in the case of carrots, spinach and tomatoes, among others [32, 36, 38, 39]. This information gives evidence that the lipid content of the algae under study did not show a greater impact on caloric contribution, as indicated in Table 1 for each of them, since calories were delivered primarily by carbohydrates and protein in *M. pyrifera*, *C. fragile* and *G. chilensis*. What we propose is to allow the use and incorporation of these algae in diet food intended to help in losing weight.

3.3 Carbohydrates

Table 1 also describes the high content of carbohydrates contributed by each of the algae under study, with values ranking from 66.1 to 75.3 g/100 g dry matter. *M. pyrifera* reaches the

VCH Auftr.-Nr. 36106 Vers.: 6.06
S:/3d/vch/_EJLST/ej_09_03/fl_0140.3d
EJLST
1. Papierl. Seite 3 – SW –
Donnerstag, den 18. 12. 08, 13 Uhr 05

Table 1. Nutritional composition† of Codium fragile, Gracilaria chilensis and Macrocystis pyrifera.

Specie	Ash [% dry weight]	Protein	Lipid	Carbohydrates††	Calories
C. fragile	20.9 ± 0.2	10.8 ± 0.0	1.5 ± 0.0	66.8 ± 0.4	323.9
G. chilensis	18.9 ± 0.1	13.7 ± 0.2	1.3 ± 0.0	66.1 ± 1.2	330.9
M. pyrifera	10.8 ± 0.3	13.2 ± 0.0	0.7 ± 0.1	75.3 ± 0.2	360.3

† Means of six analyses ± standard deviation.
†† Obtained by difference: 100% – (%ash + %proteins + %lipid), and includes dietary fiber.

highest concentration followed by *C. fragile* (66.8%), and *G. chilensis* has the lowest contribution. The values obtained in the carbohydrate investigation for the three algae are above the contents reported by several authors for fruits and vegetables daily consumed; they only resemble the carbohydrate content in dried fruit [32]. The high carbohydrate content is a very marked characteristic in most algae, comprising mainly soluble carbohydrates, sugars, including pectins, plus a lot of alginic acid in *M. pyrifera* and agar and carrageenan in *G. chilensis* [40–42].

On the other hand, it is known that algae have a high content of soluble and insoluble fiber, to which beneficial effects have been attributed with regard to diseases such as obesity and diabetes [43, 44]. This fiber is mainly composed of fiber-soluble polysaccharides that differ physically and chemically from the fiber of terrestrial plants and thus induce different physiological effects. A good use, at industrial level, of the high nutritional contribution of these three species of seaweed, constituted by minerals, proteins and carbohydrates together with the low content in fat and calories, would give developments of new products capable of reducing the risk of suffering from chronic non-transmissible diseases such as cardiovascular diseases, hypertension, diabetes and obesity, among others, so usual in our western life style. This is opposed to what is experienced in the eastern countries (Japan, China and Korea) where seaweeds have been included as ingredients in various preparations on account of their healthy content of nutrients. Seaweeds constitute a significant source of non-traditional foods which should be considered.

3.4 Amino acid composition

The amino acid composition of the three algae under study, expressed as mg/100 g of dried seaweed, is shown in Table 2, which shows the presence of all the essential amino acids in the algae analyzed. There were different levels of amino acids: In the case of *C. fragile*, the values ranged from 0.5 to 1417.7 mg/100 g dry weight. In *G. chilensis* they ranged from 0.5 to 1879.6 mg/100 g dried seaweed, and the range registered for *M. pyrifera* was 0.8–1827.3 mg/100 g dry weight. *G. chilensis* had the highest content of total amino acids, 8178.4 mg/100 g dry food, followed by *C. fragile* with a similar content to

Table 2. Amino acid composition† of Codium fragile, Gracilaria chilensis and Macrocystis pyrifera.

Amino acid [mg/100 g dry seaweed]	*C. fragile*	*G. chilensis*	*M. pyrifera*
Asp	823.1 ± 10.5	1101.5 ± 10.4	1338.8 ± 22.8
Glu	1088.8 ± 27.2	1547.3 ± 12.8	1827.3 ± 15.4
Ser	510.7 ± 9.1	749.4 ± 9.6	830.9 ± 9.6
His	95.9 ± 4.3	1124.6 ± 11.0	161.9 ± 6.1
Gly	536.6 ± 13.8	410.7 ± 4.9	664.9 ± 8.7
Thr	586.1 ± 9.7	643.9 ± 5.1	735.4 ± 6.9
Arg	420.3 ± 8.4	596.4 ± 3.7	944.7 ± 10.1
Ala	630.8 ± 13.2	663.9 ± 9.4	643.8 ± 13.7
Pro	0.5 ± 0.1	0.5 ± 0.0	0.8 ± 0.1
Tyr	389.4 ± 7.6	389.4 ± 4.6	425.9 ± 9.4
Val	1417.7 ± 17.5	765.9 ± 5.8	1140.2 ± 12.5
Met	946.7 ± 10.3	1879.6 ± 12.1	1111.6 ± 10.8
Cys	159.7 ± 2.9	756.3 ± 6.0	228.1 ± 8.3
Ile	401.8 ± 8.2	803.0 ± 5.3	507.0 ± 9.7
Leu	730.4 ± 15.4	458.8 ± 4.9	339.4 ± 13.0
Phe	476.2 ± 11.1	1087.7 ± 9.5	589.5 ± 6.7
Lys	544.6 ± 8.9	658.6 ± 8.1	321.3 ± 9.2
Total	5359.1 ± 88.3	8178.4 ± 67.8	5134.4 ± 83.2

† Means of six analyses ± standard deviation.

M. pyrifera which recorded the lowest content of amino acids. The proteins in the three types of seaweed contained high levels of essential amino acids, especially regarding the essential amino acids in *G. chilensis* (59.7% of total proteins), with prevalence of methionine, glutamic acid and histidine. On the other hand, *C. fragile* also stood out with a high level of essential amino acids (49.6% of the total protein): valine, glutamic acid and methionine. For *M. pyrifera*, the content of essential amino acids corresponding to 38.9% of total protein showed a greater contribution of glutamic acid, aspartic acid and valine [45]. In the case of the three algae under study, it was determined that proline was the amino acid lowest in concentration (according to OMS/FAO standards).

The amino acid profile shown by these species is outstanding in containing essential elements for a variety of spe-

VCH Auftr.-Nr. 36106 Vers.: 6.06
S:/3d/vch/_EJLST/ej_09_03/fl_0140.3d
EJLST
1. Papierl. Seite 4 – SW –
Donnerstag, den 18. 12. 08, 13 Uhr 05

cies, such as alanine, leucine and lysine, and non-essential ones such as glutamic acid and aspartic acid, regarded as a source of protein for the supplementary feeding of various marine species [46], which makes their use interesting because it covers the protein requirements for optimal growth of some fish. Thus, these algae could be used as partial sources of these nutrients, particularly for herbivorous fish such as siganids [47–52].

3.5 Fatty acid profiles

Algae have a low fat content, which is, however, of good quality because of high levels of unsaturated fatty acids in most of them, compared with terrestrial vegetables commonly used [53]. They may be of interest at the industrial level due to the great diversity of seaweed species distributed along the Chilean coast. Table 3 shows the composition of fatty acids contained in the seaweeds *C. fragile*, *G. chilensis* and *M. pyrifera*; in the three samples under study, the most abundant monounsaturated fatty acid (MUFA) was 18:1*n*-9*c* (oleic acid), which amounted to a contribution of 29.02% for *G. chilensis*, 19.64% for *M. pyrifera* and a contribution of 12.25% for the leaves of *C. fragile*. In addition, the latter alga showed a significant contribution of the PUFA 18:3*n*-3 (24.56%) and *M. pyrifera* had a high content of linoleic acid (18:2*n*-6) reaching values of 43.41%. The presence of C_{18} PUFA is important in human nutrition and for fish, which are unable to synthesize them; they can only be synthesized by plants, both terrestrial and marine, and they are excellent sources of *n*-3 fatty acids with 18 and 20 or more carbons (EPA and DHA) [53, 54]. However, fish can elongate and desaturate dietary fatty acids (18:2*n*-6 and 18:3*n*-3) [55].

The predominant saturated fatty acid in the three species of algae of the study was palmitic acid (16:0); in green algae, it corresponded to 17.74%, in red algae to 21.84%, and in brown algae to 16.17%, as shown in Table 3. Furthermore, the *n*-6/*n*-3 ratio, which as currently recommended by the OMS should not be higher than 10 in the diet as a whole, was at most 4–6, so that the seaweeds under study here may be of use for the reduction of the *n*-6/*n*-3 ratio [56]. Variations in fatty acid contents are attributable both to environmental and genetic differences [57]. In this study, *C. fragile* showed a lower content of MUFA than *M. pyrifera* and *G. chilensis* (19.25, 25.17 and 41.49%, respectively) and the PUFA values were 21.3% for *G. chilensis*, 36.57% for *C. fragile* and 51.41% for *M. pyrifera*, with linoleic acid being the main PUFA in this last alga [58].

Table 3. Fatty acid composition† of Codium fragile, Gracilaria chilensis and Macrocystis pyrifera.

Fatty acid	Methyl ester [%]		
	C. fragile	*G. chilensis*	*M. pyrifera*
SFA			
12:0	0.30 ± 0.01	0.35 ± 0.02	–
14:0	1.04 ± 0.05	2.05 ± 0.06	0.73 ± 0.01
15:0	0.66 ± 0.01	0.43 ± 0.01	0.36 ± 0.01
16:0	17.74 ± 0.09	21.84 ± 0.10	16.17 ± 0.06
17:0	1.11 ± 0.12	0.47 ± 0.00	–
18:0	17.38 ± 0.04	9.17 ± 0.05	3.05 ± 0.11
20:0	0.82 ± 0.00	0.70 ± 0.02	0.59 ± 0.09
22:0	2.52 ± 0.06	0.77 ± 0.09	1.30 ± 0.16
24:0	1.91 ± 0.01	0.93 ± 0.04	0.58 ± 0.18
MUFA			
14:1	–	0.15 ± 0.00	–
15:1	1.51 ± 0.03	–	0.72 ± 0.02
16:1	2.30 ± 0.02	5.84 ± 0.13	0.99 ± 0.01
17:1	–	1.89 ± 0.06	–
18:1*n*-9*t*	3.02 ± 0.01	1.58 ± 0.03	0.94 ± 0.02
18:1*n*-9*c*	12.25 ± 0.10	29.02 ± 0.18	19.64 ± 0.08
18:1*n*-7*c*	0.87 ± 0.03	3.01 ± 0.02	1.23 ± 0.01
20:1	–	–	0.91 ± 0.00
22:1	–	–	0.74 ± 0.00
PUFA			
16:2	–	–	1.19 ± 0.08
18:2	–	8.14 ± 0.01	0.39 ± 0.00
18:2*n*-6	6.24 ± 0.18	9.65 ± 0.09	43.41 ± 0.39
18:3*n*-3	24.56 ± 0.24	0.68 ± 0.01	5.45 ± 0.00
18:4*n*-3	1.11 ± 0.02	1.00 ± 0.00	–
20:4*n*-6	2.56 ± 0.01	0.53 ± 0.02	0.50 ± 0.05
20:5*n*-3	2.10 ± 0.00	1.30 ± 0.01	0.47 ± 0.01
Ratio *n*-6/*n*-3	0.32	3.42	7.42
ni	0.0	0.5	0.64

SFA, saturated fatty acid; ni, not identified.
† Means of six analyses ± standard deviation.

3.6 Tocol content

Algae are a source of vitamins (liposoluble and hydrosoluble) available all along the Pacific coast, yet not usually consumed, fresh or dried. The contents of tocols indicated in Table 4 for the three algae under study were significantly high in relation to the lipid fraction of the daily consumption of vegetable oils; only the contents of tocols in *G. chilensis* are similar to the low levels of tocols in grape seed oil, high-oleic acid sunflower seed oil, sesame seed oil and safflower seed oil. On the other hand, *C. fragile* and *M. pyrifera* recorded higher contributions, similar to those of palm oil, arachis oil, soybean oil and sunflower seed oil [59–61]. The importance of tocols is that α-, β-, γ-, and δ-tocopherol and their isomers α-, β-, γ-, and δ-tocotrienols are important liposoluble metabolites synthesized by plant cells with antioxidant action, and in humans α-tocopherol acts as vitamin E. The results of the determination of the total tocols in *C. fragile*, *G. chilensis* and *M. pyrifera* ranged between 391.9 and 1617.6 μg/g lipids, reaching the highest content in *C. fragile* where δ-tocopherol and γ-tocotrienols were considered the main ones. This high content may be associated with the higher amount of *n*-3 fatty acids. A similar

VCH Auftr.-Nr. 36106 Vers.: 6.06
S:/3d/vch/_EJLST/ej_09_03/fl_0140.3d
EJLST
1. Papierl. Seite 5 – SW –
Donnerstag, den 18. 12. 08, 13 Uhr 05

Table 4. Composition† of tocols and carotenoids of the macroalgae *Codium fragile*, *Gracilaria chilensis* and *Macrocystis pyrifera*.

	C. fragile	*G. chilensis*	M. pyrifera
Tocols		[μg/g lipid]	
α-Tocopherol	453.5 ± 3.2	86.5 ± 2.7	1327.7 ± 4.4
β-Tocopherol	56.9 ± 1.1	32.4 ± 1.3	7.7 ± 0.5
γ-Tocopherol	63.5 ± 1.9	9.5 ± 0.9	88.9 ± 4.0
γ-Tocotrienol	365.9 ± 2.3	263.5 ± 4.8	25.2 ± 1.4
δ-Tocopherol	677.8 ± 1.8	–	7.7 ± 1.1
Total	1617.6 ± 10.3	391.9 ± 9.7	1457.2 ± 11.4
Carotenoids		[μg/g dry algae]	
Lutein	0.7 ± 0.1	2.0 ± 0.3	0.3 ± 0.0
β-Carotene	197.9 ± 1.8	113.7 ± 1.3	17.4 ± 1.0
Total	198.6	115.7	17.7

† Mean of six analyses ± standard deviation.

tocol contribution showed *M. pyrifera* (1457.2 ± 11.4 μg/g lipids), mainly α-tocopherol. The two values differ clearly from the low levels identified in *G. chilensis* whose main component was γ-tocotrienol, with a value of 263.5 ± 4.8 μg/g lipids. Despite the low lipid content in these algal species, the values of vitamin E are relevant, especially in the case of *C. fragile* and *M. pyrifera*, as they contribute to the stability of the PUFA present in these algae, preventing the formation of free radicals and thus converting these algae into good complementary foods in view of the significant contribution of vitamin E and PUFA.

3.7 Carotenoid contents

The analysis of the carotenoids carried out on the three algae, detailed at the bottom of Table 4, showed significant concentrations of two carotenoids: lutein and β-carotene, the latter being the predominant carotenoid for the three algae, with concentrations of 197.9, 113.7 and 17.4 μg/g dried seaweed, respectively. The values of β-carotene both in *C. fragile* and in *G. chilensis* exceed those found in fresh carrot (88.33 μg/g) and other vegetables of mass consumption like spinach, cabbage, Swiss chard, averaging 25 μg/g [62, 63]. *G. chilensis* supplied the greatest contribution of lutein by the algae under consideration with a value of 2.0 μg/g dried seaweed, and *M. pyrifera* the lowest, as shown in Table 4. There are no significant differences between these values and those contributed by usually consumed vegetables [64].

According to the literature, the most appropriate dose of β-carotene intake is 15 mg/day, while for lutein it is 6 mg/day to contribute to a healthy state, and consuming 75 g of *C. fragile* would complement the daily recommended requirements of β-carotene [65].

4 Conclusions

The seaweeds *C. fragile*, *G. chilensis* and *M. pyrifera* examined in the study have high ash contents that can contribute with important microelements to human and animal nutrition. In addition, they present valuable protein contents and relatively high levels of essential amino acids, similar to cereals like wheat, corn and oats and superior to food used in animal feed as straw, and similar to hay. On the other hand, the carbohydrates include soluble sugars that can be used by humans and animals, together with considerable quantities of alginic acid and carrageenans which might act as dietetic fiber. The low total lipid contents of seaweeds is similar to other foods (cereals as rice and rye, and legumes as bean, chick-pea and beans) that have less than 2% of fat, but with the advantage that the fat material of the algae possesses a suitable PUFA *n*-6/*n*-3 relation of high effect in human and animal nutrition. Equally, the α-tocopherol and β-carotenoid contents in these seaweeds are beneficial because they can act as vitamins and antioxidants. Nevertheless, there exists an increasing need to extend the researches in this area like, for example, in the identification of the type of essential elements and iodine content besides the content of the sterols present in these algae.

Acknowledgments

This study was funded by Dirección de Investigación y Desarrollo DID, Universidad de Chile under Project DID SAL 02/14-2. We also thank engineer Oscar Alcalde (Manager Director of Cultivos Caldera, Caldera, Chile) for supplying fresh and dehydrated seaweed samples.

Conflict of interest statement

The authors have declared no conflict of interest.

References

[1] B. Santelices: *Catálogo de las Algas Marinas Bentónicas de la Costa Temperada del Pacífico de Sudamérica*. Ed. Universidad Católica de Chile, Santiago (Chile) 1991, pp. 24–65.

[2] V. Chapman, D. Chapman: *Seaweeds and Their Uses*. 3rd Edn. Chapman & Hall, New York, NY (USA) 1980, pp. 25–42.

[3] M. Norziah, Ch. Ching: Nutritional composition of edible seaweed *Gracilaria changgi*. *Food Chem.* 2000, **68**, 69–76.

[4] J. Chan, P. Cheung, P. Ang: Comparative studies on the effect of three drying methods on the nutritional composition of seaweeds *Sargassum hemiphyllum* (Turn) C. Ag. *J Agric Food Chem.* 1997, **45**, 3056–3059.

[5] M. Lahaye: Marine algae as sources of fibers: Determination of soluble and insoluble dietary fiber contents in some sea vegetables. *J Sci Food Agric.* 1991, **54**, 587–594.

VCH Auftr.-Nr. 36106 Vers.: 6.06
S:/3d/vch/_EJLST/ej_09_03/fl_0140.3d
EJLST
1. Papierl. Seite 6 – SW –
Donnerstag, den 18. 12. 08, 13 Uhr 05

[6] M. Dreher: *Handbook of Dietary Fiber.* Marcel Dekker, New York, NY (USA) 1987, pp. 31–56.

[7] A. Stephen, J. Cummings: Mechanism of action of dietary fiber in the human colon. *Nature*. 1980, **24**, 283–284.

[8] D. Kritchevsky: Dietary fiber. *Annu Rev Nutr.* 1988, **8**, 301–328.

[9] R. Johns, P. Nichols, G. Perry: Fatty acid composition of ten algae from Australian waters. *Phytochemistry.* 1979, **18**, 799–802.

[10] V. Mishra, F. Temelli, P. Ooraikul, J. Craigie: Lipids of the red algae *Palmaria palmate. Botanica Marina.* 1993, **36**, 169–174.

[11] S. Khotimchenko, V. Vaskovsky, T. Titlyanova: Fatty acids of marine algae from the Pacific coast of North California. *Botanica Marina.* 2002, **45**, 17–22.

[12] S. Kamat, S. Wahídulla, L. D'Souza, C. Naik, V. Ambiye, D. Bhakuni, A. Goel, H. Garg, R. Srimal: Bioactivity of marine organisms. VI. Antiviral evaluation of marine algal extracts from the Indian coast. *Botanica Marina.* 1992, **35**, 161–164.

[13] C. Bird, J. McLachlan, E. Oliveira: *Gracilaria chilensis* sp. nov. (Rhodophyta, Gigartinales), from Pacific South America. *Can J Bot.* 1987, **64**, 2928–2934.

[14] SERNAPESCA: *Datos Estadísticos del Desembarque, Producción y Cosecha de Algas Marinas Chilenas.* Ed. Servicio Nacional de Pesca, Santiago (Chile) 2003, pp. 27–32.

[15] B. Santelices, F. Ojeda: Effects of canopy removal on the understory algal community structure of coastal forests of *Macrocystis pyrifera* from southern South America. *Mar Ecol Prog Ser.* 1984, **14**,165–173.

[16] Producción de harina de la macroalga *Macrocistis pyrifera* y su utilización como ingrediente para la alimentación de salmónidos en la region de Magallanes y Antártica Chilena. Proyecto Fondecyt N°DO1I1164. *Boletín de la Comisión Nacional de Ciencia y Tecnología*, Santiago (Chile) 2001, pp. 231–233.

[17] P. Neil, O. Alcalde, S. Fougeron, S. Navarrete, J. Correa: Invasion of *Codium fragile* ssp. *tomentosoides* in Northern Chile: A new threat for *Gracilaria* farming. *Aquaculture.* 2006, **259**, 202–210.

[18] K. Hahn: Nutrition and growth of abalone. In: *Handbook of Culture of Abalone and other Marine Gastropods.* Ed. K. O. Hahn, CRC Press, Boca Raton, FL (USA) 1989, pp. 135–156.

[19] A. Fleming, R. Van Berneveld, P. Hone: The development of artificial diets for abalone: A review and future directions. *Aquaculture*. 1996, **140**, 5–53.

[20] AOAC: *Official Methods of Analysis of Association of Official Analytical Chemists International. Vol. I, II.* 16th Edn. AOAC, Gaithersburg, MD (USA), 1995, p. 870.

[21] W. Christie: *Gas Chromatography and Lipids, a Practical Guide*. 3rd Edn. The Oily Press, Ayr (UK) 1992, pp. 63–66.

[22] M. Alaiz, J. Navarro, J. Girón, E. Vioque: Amino acid analysis by high-performance liquid chromatography after derivatization with diethyl ethoxymethylenemalonate. *J Chromatogr A.* 1992, **591**, 181–186.

[23] Asociación Española de Normalización: Norma UNE 55037-73. *Catálogo de Normas UNE*, Madrid (Spain) 1991, pp. 23–26.

[24] AOCS: *Official Methods and Recommended Practices of the American Oil Chemists Society.* 4th Edn. AOCS Press, Champaign, IL (USA) 1993, pp. 54–56.

[25] L. Henry, G. Catignani, S. Schwartz: The influence of carotenoids and tocopherols on the stability of safflower seed oil during heat-catalyzed oxidation. *J Am Oil Chem Soc*. 1998, **75**, 1399–1402.

[26] J. Fleurence: Seaweed proteins: Biochemical nutritional aspects and potential uses. *Trends Food Sci Technol.* 1999, **10**, 25–28.

[27] G. Castro, D. Carrillo, F. Pérez-Gil: Chemical composition of *Macrocystis pyrifera* collected in summer and winter and its possible use in animal feeding. *Cienc Mar.* 1994, **20**, 33–40.

[28] M. Rodríguez, C. Hernández: Seasonal and geographic variations of *Macrocystis pyrifera* chemical composition at the western coast of Baja California. *Cienc Mar.* 1991, **17**, 91–107.

[29] N. Pak, H. Araya: Macroalgas comestibles de Chile como fuente de fibra dietética: Efecto de la digestibilidad aparente de proteínas, fibra, energía y peso de deposiciones en ratas. *Arch Latinoam Nutr.* 1996, **46**, 42–46.

[30] T. Murai, T. Akiyama, T. Nose: Effect of amino acid balance of efficiency in utilization of diet by fingerling carp. *Bull Jpn Soc Sci Fish* 1984, **50**, 893–897.

[31] A. Jackson, B. Capper, A. Matty: Evaluation of some plant proteins in complete diets for tilapia *Sarotherodon mossanbicus. Aquaculture*. 1982, **27**, 97–109.

[32] H. Schmidt-Hebbel, I. Pennacchiottii, L. Masson: *Tabla de Composición Química de Alimentos Chilenos.* 8th Edn. University of Chile, Santiago (Chile) 1992, pp 21–■ ■.

[33] D. Carrillo, G. Castro, F. Pérez-Gil, E. Rosales, I. Manzano: The seaweed (*Sargassum sinicola* Setchel and Gardner) as an alternative for animal feeding. *Cuban J Sci.* 1992, **26**, 177–184.

[34] H. Etcheverry, G. López: Estudios químicos en *Macrocystis pyrifera*, constituyentes inorgánicos y orgánicos. *Rev Biol Mar.* 1982, **18**, 73–79.

[35] A. Jiménez, I. Goñi: Nutritional evaluation and physiological effects of edible seaweeds. *Arch Latinoam Nutr.* 1999, **49**, 114–120.

[36] S. Carrillo, M. Casas, F. Ramos, F. Pérez-Gil, I. Sánchez: Algas marinas de Baja California Sur, México: Valor nutrimental. *Arch Latinoam Nutr.* 2002, **52**, 400–405.

[37] D. Sánchez-Machado, J. López-Cervantes, J. López-Hernández, P. Paseiro-Losada: Fatty acids, total lipid, protein and ash contents of processed edible seaweeds. *Food Chem.* 2004, **85**, 439–444.

[38] M. Chavez, V. Chávez, A. Roldán, S. Ledesma, M. Mendoza, F. Pérez-Gil, C. Hernández, F. Chaparro: *Tablas de Valor Nutritivo de los Alimentos de Mayor Consumo en Latinoamerica. Edición Internacional.* Instituto Nacional de la Nutrición, Instituto Nacional de Cancerología, Editorial Pax (México) 1996, pp. 98–102.

[39] F. Herbetreau, L. Coifford, A. Derrien, Y. De Roeck-Holzhauer: The fatty acid composition of five species of macroalgae. *Botanica Marina.* 1997, **40**, 25–27.

[40] D. Hong, H. Hien: Nutritional analysis of Vietnamese seaweeds for food and medicine. *Biofactors.* 2004, **22**, 323–325.

[41] H. Englyst, M. Quigley, G. Hudson: Definition and measurement of dietary fibre. *Eur J Clin Nutr.* 1995, **3 Suppl**, 48–62.

[42] C. Bulboa, J. Macchiavello, E. Oliveira, E. Fonck: First attempt to cultivate the carrageenan-producing seaweed *Chondracanthus chamissoi* (C. Agardh) Kützing (Rhodophyta; Gigartinales) in North Chile. *Aquac Res.* 2005, **36**, 1069–1074.

[43] P. Burtin: Nutritional value of seaweeds. *Electron J Environ Agric Food Chem*. 2003, **2**, 498–503.

VCH Auftr.-Nr. 36106 Vers.: 6.06
S:/3d/vch/_EJLST/ej_09_03/fl_0140.3d
EJLST
1. Papierl. Seite 7 – SW –
Donnerstag, den 18. 12. 08, 13 Uhr 05

[44] N. Pak: Fibra dietética: Concepto, contenido en alimentos y consumo en Chile. *Rev Chil Nutr.* 1992, **20**, 124–135.

[45] M. Wahbeh: Amino acid and fatty acid profiles of four species of macroalgae from Aqaba and their suitability for use in fish diets. *Aquaculture*. 1997, **159**, 101–109.

[46] W. Neg, K. Wee: The nutritive value of cassava leaf meal in pelleted feed for Nile tilapia. *Aquaculture*. 1989, **83**, 45–58.

[47] K. Jouncey: The effects of varying protein level on the growth, food conversion, protein utilization and body composition of juvenile tilapia. *Aquaculture*. 1982, **27**, 43–54.

[48] K. Kesamura, N. Okumura, H. Takeda, A. Kuroki: Studies on the nutrition of *Tilapia nilotica*. III. Protein requirements of fingering *Tilapia nilotica*. *Bull Faculted Agric Miyazaki Univ (Japan)*. 1982, **29**, 285–291.

[49] S. De Silva, W. Perrara: Effects of dietary protein level on growth, food conversion and protein use in young *Tilapia niotica* at four salinities. *Trans Am Fish Soc*. 1985, **114**, 584–589.

[50] A. Tacon, C. Cowey: Protein and amino acid requirements. *Fish Energetic New Perspect*. 1985, **16**, 155–183.

[51] M. Millikin: Qualitative and quantitative nutrient requirements of fishes: A review. *Fish Bull.* 1982, **80**, 655–696.

[52] S. Bowen: Dietary protein requirement of fish. A reassessment. *Can J Fish Aquac Sci.* 1987, **44**, 1995–2001.

[53] B. Darcy-Vrillon: Nutritional aspects of the developing use of marine macroalgae for human food industry. *Int J Food Sci Nutr.* 1993, **44**, 23–25.

[54] J. Araya: *Riesgos y Beneficios del Consumo de Grasas y Aceites.* Departamento de Nutrición, Facultad de Medicina, Universidad de Chile, Santiago (Chile) 2007, pp. 38–42.

[55] C. Cowey: Use of synthetic diets and biochemical criteria in the assessment of nutrients of fish. *J Fish Res Board Can.* 1976, **33**, 1040–1045.

[56] L. Mahan, K. Escott-Stump: *Nutrición y Dietoterapia, de Krause*. 10th Edn. McGraw-Hill Interamericana, Madrid (Spain) 2001, pp. 23–25.

[57] M. Nelson, C. Phleger, P. Nichols: Seasonal lipid composition in macroalgae of the Northeastern Pacific Ocean. *Botanica Marina*. 2002, **5**, 58–65.

[58] S. Khotimchenko, V. Vaskovsky, T. Titlyanova: Fatty acids of marine algae from the Pacific coast of North California. *Botanica Marina*. 2002, **45**, 17–22.

[59] M. Honya, T. Kinoshita, M. Ishikawa, H. Mori, K. Nisizawa: Seasonal variation in the lipid content of cultured *Laminaria japonica*; fatty acids, sterols, β-carotene and tocopherol. *J Appl Phycol.* 1994, **6**, 25–29.

[60] P. Harris, N. Embree: Quantitative consideration of the effect of polyunsaturated fatty acid content of the diet upon requirements for vitamin E. *Am J Clin Nutr.* 1963, **13**, 385–392.

[61] ■ author, editor?■ : *Codex Alimentarius. Normas del Codex para Aceites Vegetales Especificados.* ■ publisher■ , ■ city, country■ Enmendado 2003–2005, pp. 1–15.

[62] G. Gayathri, K. Platel, J. Prakash, K. Srinivasan: Influence of antioxidant spices on the retention of β-carotene in vegetables during domestic cooking processes. *Food Chem*. 2004, **84**, 35–43.

[63] S. Macías de Costa, M. Montenegro, T. Arregui, M. Sánchez de Pinto, M. Nazareno, B. López de Mishima: Caracterización de acelga fresca de Santiago del Estero (Argentina). Comparación del contenido de nutrientes en hojas y tallo. Evaluación de los carotenoides presentes. *Cienc Tecnol Alimentos.* 2003, **23**, 33–37.

[64] D. Rodríguez-Amaya: *A Guide to Carotenoid Analysis in Foods.* Departamento de Ciencia de Alimentos, Facultad de Ingeniería de Alimentos, Universidad Estatal de Campinas (Brazil) 2001, pp. 32–35.

[65] N. Krinsky: The antioxidant and biological properties of the carotenoids. *Annu Acad Sci NY (USA)*. 1998, **854**, 443–447.

VCH Auftr.-Nr. 36106 Vers.: 6.06
S:/3d/vch/_EJLST/ej_09_03/fl_0140.3d
EJLST
1. Papierl. Seite 8 – SW –
Donnerstag, den 18. 12. 08, 13 Uhr 05