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Evaluation of Marine Algae Wakame (*Undaria pinnatifida*) and Kombu (*Laminaria digitata japonica*) as Food Supplements

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Summary

Crude proteins and their amino acid composition, β-carotene, vitamins B_1, B_2, B_6, niacin and minerals were determined in two edible brown marine algae (*Phaeophyceae*), Wakame (*Undaria pinnatifida*) and Kombu (*Laminaria digitata japonica*). The amino acid scores for five key essential amino acids, frequently deficient in mixed human diet, and essential amino acid index were calculated. The results have shown the presence of all essential amino acids. The values of essential amino acid ratios of analysed algae exceed the ratios of reference proteins suggested by FAO/WHO/UNU, except for tryptophan, the first limiting amino acid in both analysed algae. Iodine, the most important component of sea vegetables is present in high amounts as well as the vitamins B_1, B_2, B_6, niacin and β-carotene. The content of minerals was found high, while the presence of heavy metals was negligible.

Key words: Wakame, Kombu, protein quality, vitamin and mineral composition

Introduction

Edible marine algae, also called sea vegetables, are plants that grow in the sea. East Asian population (Japan, Korea, China) have regularly utilised the sea vegetables since ancient times. The result of such a diet is very high iodine consumption and extremely low incidence of breast cancer (*1*). In Europe, as a contrast, there is a considerable lack of interest for their consumption, and consequently the cultivation of autochtonous algae is negligible (*2*).

The macrobiotic diet, which came to Europe from Japan, contributed to the introduction of sea vegetables in our staple diet. It appeared especially in more developed postindustrial countries, where voluntary vegeterian and macrobiotic diets are more and more popular (*1,3*) and where, because of the increasing interest, marine algae are imported in dried state (*2*). Nevertheless, with exception of France, there are no European specific regulations concerning their utilisation for human consumption (*4,5*).

Undaria pinnatifida and *Laminaria digitata japonica* (commercial names Wakame and Kombu, respectively) are the most utilised brown algae (*Phaeophyceae*) in macrobiotic diet, where these algae are consumed daily, although in small quantities (*6*). Their chemical composition indicates that Wakame and Kombu are valuable ingredients to any diet (*7,8*), but they are also of particu-

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lar nutritional importance to vegans and vegeterians, because together with legumes and grains they represent the supplementary source of proteins (*1*).

When compared to land plants marine algae have 10–20 times higher content of vitamins (*9*) and represent an excellent source of vitamins A, D, E, B-complex and B_{12} (*1,7*), although the biodisponibility of algal vitamin B_{12} in humans is contradictory (*10*).

Sea vegetables are an excellent source of minerals due to their capacity to absorb inorganic substances from the ocean by polisaccharidic structure of their surface. Mineral composition and mineral contents are the functions of environmental, geographical and physiological factors (*2,4*). Mineral content is higher than that in edible land plants and animal products (*4*), and that is why the sea vegetables are effective in relatively small supplementary amounts and can supply our food with many missing elements (*9*).

The carbohydrates in *Phaeophyceae*, containing soluble fibres of alginates, laminarans, fucans and insoluble cellulose, are indigestible by man and therefore considered dietary fibres. They differ chemically and physicochemically from those in land plants. From nutritional point of view, they provide the bulk in faeces and hold water (*5,11*). Due to their polysaccharidic structure they bind metal ions (*5,12*), and can remove heavy metals from our bodies through faeces (*1*).

Lipid content in sea vegetables is very low, ranging from 1–5 % of dry matter (*8,13*). Nevertheless algal lipids have a higher proportion of unsaturated fatty acids than land plants (*2*). Furthermore, marine algae possess antibiotic and anticarcinogenic activity (*2,14*). They contain lignans that are readily converted by intestinal microflora to non-steroidal estrogenic molecules, which explains their therapeutic and preventive effect against estrogen-driven neoplasms (*1*). The aim of this work was to evaluate Wakame and Kombu as natural and useful food supplements in our western staple diet.

Material and Methods

Wakame and Kombu imported from Japan were purchased in dried state in a local health store. The samples were pulverised and crude proteins determined according to AOAC (*15*). Minerals were determined by atomic absorption spectrophotometry according to Rowe (*16*). Mercury and arsenic were determined by vapor generating technique with atomic absorption spectrophotometry (*17*). Iodine was determined according to AOAC (*15*). Phosphorus and β-carotene were determined by spectrophotometric method, vitamin B_1 by thiochrom method, vitamin B_2 by fluorometric method, all according to AOAC (*15*), while vitamin B_6 and niacin were determined by HPLC method (*18,19*).

Amino acid analyses

Chemicals

4-Dimethylaminoazobenzene-4´-sulphonyl chloride (DABS-CI) was purchased from Fluka (Buchs, Switzerland). A standard calibration mixture of amino acids of the highest grade and 6 mol/L hydrochloric acid (constant boiling, sequanal grade) were obtained from Pierce (Pierce Chemical Co., Rockford, IL). Acetonitrile, methanol, acetone and ethanol of HPLC grade and NaOH were obtained from Carlo Erba (Farmitalia, Italy). Analytical reagent grade potassium dihydrogen phosphate was purchased from Merck (Darmstadt, Germany). Triple-distilled water was prepared in the laboratory and used for the preparation of buffers. The buffer A used for the HPLC analyses was filtered throught a 0.22-μm Millipore filter (Millipore Corp., Milford, MA).

Acid hydrolysis with HCl (*c*=6 mol/L)

The pyrex hydrolysis tubes (10 cm x 7 mm i.d.) for high sensitivity amino acid analysis were freed from amino acid contaminations by heating at 600 °C overnight. Hydrolysis was carried out as follows: 10 mg of each dried and powdered sample were placed into a pyrex tube, treated with 1 mL of 6 mol/L hydrochloric acid (constant boiling, sequanal grade), sealed under vacuum and left at 110 °C for 22 h. The volume of 20 μL of the hydrolysed sample was pipetted into a test tube, dried under vacuum and derivatized (*20*).

Alkaline hydrolysis with NaOH

Alkaline hydrolysis, using 4.2 mol/L NaOH, was performed to check the recovery of tryptophan in protein samples with high sugar concentration. The hydrolysis was performed according to the Hugli and Moore method (*21*).

Preparation of DABS-derivatives

DABS-CI solution (4 nmol/μL acetonitrile) was prepared according to the method by Chang *et al.* (*22*) with slight modifications (*20,23*). Dried samples were resuspended in 50 μL of 0.2 mol/L sodium hydrogen carbonate, pH=9 and treated with 100 μL of DABS-CI solution (4 nmol/μL acetonitrile). The mixture was left at 70 °C for 10 min and then dried under vacuum. The residue was redissolved in a proper volume of 70 % ethanol and 20 μL of this volume were used for the determination of amino acids.

HPLC analysis

A Gold liquid chromatographic system from Beckman (Beckman, Berkeley, CA) was used for amino acid analyses. The HPLC apparatus consisted of two Model 126 pumps, a PC-8300 solvent programmer, a Model 210 sample injection valve, a 20-μL injection loop, and a Model 166 variable wavelength UV-visible range detector, equipped with a 12-μL flow cell. Integration of peak areas was obtained by means of Gold software. Separation of DABS-amino acids was performed using a 3-μm Supelcosil LC-18 T column (15 cm × 4.6 mm i.d.) protected with a 5-μm Supelcosil LC-18 T guard column (2 cm × 4.6 mm i.d.) obtained from Supelco (Supelco, Bellefonte, PA). Solvent A contained 25 mM potassium dihydrogen phosphate buffer, pH=7.05 and solvent B contained acetonitrile and methanol (70:30). The gradient was: 1 min at 20 % of solvent B, 5 min up to 25 % of solvent B, 14 min at 25 % of solvent B, 1 min up to 32 % of solvent B, 6 min at 32 % of solvent B, 7 min up to 40 % of solvent B, 9 min up to 60 % of solvent B, 1 min up to 75 % of solvent B, 5 min at 75 % of solvent B. The gradient was then returned to 20 % of solvent B. The flow rate was 1.5 mL/min and detection was performed at 436 nm.

Results and Discussion

Results are shown in Tables 1–7. All the data in Tables 1, 4 and 6 are estimated as mean values with N=3 replicants, using confidence limits for means at $p \leq 0.05$ statistical significance.

In macrobiotic diet the intake of sea vegetables is 5 % or less of total daily amount of food (*6*), while recent recommendations are no more than 2–4 g of dry algae (personal communication). For the western diet it is recommended to consume about 29 g of algae a week as a food supplement (*1*) *i.e.* about 4 g a day, which is in agreement with the recent macrobiotic recommendations.

Table 1. Crude protein contents in g/100 g d.w., and the amino acid composition in mg/g d.w. of marine algae Wakame (*Undaria pinnatifida*) and Kombu (*Laminaria digitata japonica*)

Crude protein	Wakame	Kombu
$(N \times 6.25)/w/(g/100\ g)$	16.3 ± 1.4	6.2 ± 0.4
Amino acid	w/(mg/g)	
Aspartic acid	10.18 ± 1.27	4.69 ± 0.43
Glutamic acid	10.65 ± 0.57	3.86 ± 0.11
Serine	5.76 ± 0.08	2.45 ± 0.20
*Threonine	7.33 ± 0.57	3.41 ± 0.46
Glycine	8.76 ± 0.87	3.31 ± 0.41
Alanine	27.20 ± 0.70	4.51 ± 0.63
Arginine	8.41 ± 1.17	2.96 ± 0.23
Proline	5.52 ± 0.40	1.91 ± 0.14
*Valine	16.84 ± 0.32	6.01 ± 0.76
*Methionine	3.58 ± 0.42	1.49 ± 0.11
*Isoleucine	7.91 ± 0.18	2.61 ± 0.35
*Leucine	13.70 ± 1.36	4.45 ± 0.09
*Tryptophan	0.43 ± 0.02	0.19 ± 0.01
*Phenylalanine	7.80 ± 0.17	2.82 ± 0.07
Cystine	2.41 ± 0.17	1.96 ± 0.17
*Lysine	11.12 ± 1.07	4.77 ± 0.23
*Histidine	5.25 ± 0.37	2.38 ± 0.14
Tyrosine	4.31 ± 0.16	1.74 ± 0.13
Total	157.16 ± 9.87	55.46 ± 4.67

*Essential amino acids

Table 2. The essential amino acid ratios of Wakame (*Undaria pinnatifida*) and Kombu (*Laminaria digitata japonica*) compared with FAO/WHO/UNU suggested pattern of amino acid requirements in mg/g of protein

Amino acid	Wakame	Kombu	FAO/WHO/UNU reference protein
	w/(mg/100 g)		
His	32	39	19
Ile	49	42	28
Leu	84	72	66
Lys	68	77	58
Met+Cys	37	55	25
Phe+Tyr	74	73	63
Thr	45	55	34
Trp	3	3	11
Val	103	97	35
Total	495	513	339
Met	22	24	
Cys	15	31	
Phe	48	45	
Tyr	26	28	

According to the results crude protein content in Wakame and Kombu is 16.3 and 6.2 g/100 g d.w., respectively (Table 1). Moisture in Wakame and Kombu was found to be 9.5 and 7.6 %, respectively (data not reported), meaning that 4 g of Wakame or Kombu correspond to 3.62 and 3.70 g d.w., respectively, or the daily intake of Wakame or Kombu supply 0.59 and 0.22 g of proteins, respectively (Table 1).

Table 3. Amino acid scores (%) and essential amino acid index (EAAI) for Wakame (*Undaria pinnatifida*) and Kombu (*Laminaria digitata japonica*)

	Wakame (*Undaria pinnatifida*)	Kombu (*Laminaria digitata japonica*)
	scores/%	
Amino acid score	27	27
Lys-Met+Cys score	132	176
Lys-Met+Cys-Trp score	97	127
Lys-Met+Cys-Trp -Thr score	106	135
EAAI	100	100

Table 4. Vitamin contents of marine algae Wakame (*Undaria pinnatifida*) and Kombu (*Laminaria digitata japonica*) in mg/100 g d.w.

Vitamin	Wakame	Kombu
	w/(mg/100 g)	
β-carotene	1.30 ± 0.12	2.99 ± 0.09
(retinol equivalent)	0.217 ± 0.006	0.481 ± 0.015
Vitamin B_1	0.30 ± 0.04	0.24 ± 0.02
Vitamin B_2	1.35 ± 0.09	0.85 ± 0.08
Vitamin B_6	0.18 ± 0.02	0.09 ± 0.01
Niacin	2.56 ± 0.11	1.58 ± 0.14

Both analysed algae contain all essential amino acids (Table 1). Total amino acid content is 47.1 % in Wakame and 50.7 % in Kombu. The first limiting amino acid in both analysed algae is tryptophan (Table 1). Their amino acid ratios are 73 % lower (Table 2) than in the reference protein (*24*), the consequence of which is low amino acid score in both analysed algae (Table 3). The amino acid scores Lys-Met+Cys, Lys-Met+Cys-Trp and Lys-Met+Cys-Trp-Thr are in both analysed algae very high as well as the essential amino acid index (EAAI). EAAI is calculated as the geometric mean value of the ratios for 8 essential amino acids (Ile, Leu, Lys, Met+Cys, Phe+Tyr, Thr, Trp, Val) (*25*), and the EAAI above 100 is considered 100 (*26*) (Table 3). According to the results, the amino acid ratios of Lys-Met+Cys-Thr, often deficient in mixed human diet (*26*), are higher than those in the reference protein (Table 2), suggesting a good protein quality. These results should be corrected by digestibility (*26*), which is found to be rather low *in vitro* (*27*). However, it is claimed that regular consumption of sea vegetables in the diet encourages resident intestinal microflora to develop sea vegetable digestive enzymes in 4 to 6 weeks (*1*), enhancing the nutritive value of algal proteins. In macrobiotic diet Wakame is an ingredient in miso soup, Kombu is always cooked with legumes or other vegetables, while whole cereal grains can be spiced with powdered Wakame and Kombu (*6*).

Table 5. Intake of vitamins in daily recommended doses of Wakame (3.62g d.w.) and Kombu (3.70 g d.w.) and their benefit for adults between 25–50 years of age

Vitamin	*m*(recommended daily dose)/mg (*30*,*31*)	*m*(intake)/μg		Contribution/%	
		Wakame	Kombu	Wakame	Kombu
Vitamin A (retinol)	1000 μg	7.85	17.79	0.85	1.78
Vitamin B_1	1.5 mg	10.86	8.88	0.72	0.59
Vitamin B_2	1.7 mg	48.87	31.45	2.87	1.85
Vitamin B_6	2.0 mg	6.25	3.33	0.31	0.17
Niacin	19 mg	92.67	58.46	0.49	0.31

Table 6. Mineral composition of Wakame (*Undaria pinnatifida*) and Kombu (*Laminaria digitata japonica*) in mg/100 g d.w.

Minerals	Wakame	Kombu
	w/(mg/100 g)	
Ca	950 ± 30	880 ± 20
Mg	405 ± 10	550 ± 15
P	450 ± 12	300 ± 10
I	26 ± 2.4	170 ± 5.5
Na	6494 ± 254	2532 ± 120
K	5691 ± 215	5951 ± 305
Ni	0.265 ± 0.015	0.325 ± 0.020
Cr	0.072 ± 0.026	0.227 ± 0.073
Se	< 0.05	< 0.05
Fe*	1.54 ± 0.07	1.19 ± 0.03
Zn*	0.944 ± 0.038	0.886 ± 0.330
Mn*	0.332 ± 0.039	0.294 ± 0.017
Cu*	0.185 ± 0.016	0.247 ± 0.076
Pb*	0.079 ± 0.015	0.087 ± 0.021
Cd*	0.028 ± 0.006	0.017 ± 0.007
Hg	0.022 ± 0.003	0.054 ± 0.005
As	0.055 ± 0.008	0.087 ± 0.006

* Ref. (*8*)

Since the first limiting amino acid in cereals is lysine, in legumes it is methionine (*28*), in miso, the soybean fermented product, it is sulphur-containing pair methionine+cystine (*29*), the amino acid contents of Wakame and Kombu could be considered complementary (*30*).

The results in Table 4 show that Wakame and Kombu have high contents of β-carotene *i.e.* 1.30 and 2.99 mg/100 g d.w. or 217 and 481 μg retinol/100 g d.w., respectively (*30*). The benefit of daily intake of Wakame and Kombu (3.62 and 3.70 g d.w., respectively) in daily recommended doses of determined vitamins for adults between 25–50 years of age is shown in Table 5.

The basic component in sea vegetables is iodine, an essential trace element and an integral part of two hormones released by the thyroid gland (*30*). According to the results in Table 6, Wakame and Kombu contain 26 and 170 mg/100 g d.w. of iodine, respectively. The recommended daily dose for adults is 150 μg (*30*), meaning that the consumption of 557 mg of Wakame and 88 mg of Kombu would satisfy the daily requirement for iodine. The toxic dose of iodine for adults is thought to be over 2000 μg per day (*30*). The intake of recommended amount of Wakame and Kombu per day would supply 0.94 and 6.29 mg of iodine, respectively, meaning that 1.18 g of Kombu a day would not exceed the recommended safe dose of iodine. However, it is claimed that only iodine supplements can be toxic if taken in excess, while eating sea vegetables should cause no concern (*9*).

Table 7. Intake of calcium, magnesium and phosphorus in daily recommended doses of Wakame (3.62 g d.w.) and Kombu (3.70 g d.w.) and their benefit for adults between 25–50 years of age

Mineral	*m*(recommended daily dose)/mg (*31*)	*m*(intake)/mg		Contribution/%	
		Wakame	Kombu	Wakame	Kombu
Ca	800	34.39	32.56	4.3	4.1
Mg	350	14.66	20.35	4.2	5.8
P	800	16.29	11.10	2.0	1.4

Wakame and Kombu are an excellent source of calcium, magnesium and phosphorus (Table 6). The benefit of daily intake of Wakame (3.62 g d.w.) and Kombu (3.70 g d.w.) in daily recommended doses of macrominerals such as calcium, magnesium and phosphorus is shown in Table 7.

Because the diet rarely lacks sodium, the minimum sodium requirement for adults is set to 560 mg, while the limiting daily intake should be 2400 mg of sodium (equivalent to 6 g of salt) (*30*). According to the results (Table 6), the recommended daily intake of Wakame and Kombu will provide 235 mg or 9.8 % and 93.6 mg or 3.9 % of sodium, respectively. The dietary deficiency of potassium is also unlikely, so the minimum requirement for adults is estimated to be 2000 mg/day (*30*). If 3.62 g d.w. of Wakame and 3.70 g d.w.of Kombu are consumed, the daily intake of potassium would be 206 mg or 10.3 % and 220 mg or 11.0 %, respectively.

Heavy metals mercury, cadmium and lead are the most toxic for humans. The determined quantity of mercury in Wakame and Kombu (Table 6) in relation to daily intake of analysed algae is less than 0.04 mg/day, the tolerable daily intake of mercury established by FAO/WHO Expert Commitee (*32*).

The tolerable daily intake of cadmium in humans is 50–150 μg (*32*), meaning that 3.62 g d.w. of Wakame and 3.70 g d.w. of Kombu would provide 1.01 and 0.62 μg of cadmium, respectively. The limit for lead in drinking water suggested by WHO is 100 μg/L. Assuming that 2.5 L of water is consumed daily, the limit is 250 μg of lead/day (*32*). The recommended amount of Wakame and Kombu would therefore supply 2.86 and 3.22 μg of lead, respectively (Table 6).

Conclusions

High values of essential amino acid ratios except for tryptophan, the first limiting amino acid in both analysed algae, reveal a good protein quality, which is complementary in combination with proteins in other vegetables, grains and legumes.

In combination with other food, the analysed algae contribute to satisfying the recommended daily doses of vitamins especially β-carotene and vitamin B_2.

Being the excellent source of most minerals, the analysed algae can compensate for frequently low content of minerals in food plants grown on soils from which minerals have been depleted by modern agricultural methods.

Furthermore, considering that the sea vegetables eaten regularly are the best natural source of iodine, and that they represent an easy and nontoxic way to meet the daily requirement of iodine in the diet, the analysed algae consumed as food suplement can improve the nutritive value of vegeterian or omnivorous diet.

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Morske alge Wakame (*Undaria pinnatifida*) i Kombu (*Laminaria digitata japonica*) kao dodatak hrani

Sažetak

U dvjema jestivim smeđim morskim algama (*Phaeophyceae*), Wakame (*Undaria pinnatifida*) i Kombu (*Laminaria digitata japonica*), određivani su sirovi proteini i njihov aminokiselinski sastav, β-karoten, vitamini B_1, B_2, B_6, niacin i minerali. Izračunati su indeksi pet ključnih esencijalnih aminokiselina, koje često nedostaju u prehrani, te indeks esencijalnih aminokiselina. Rezultati pokazuju da alge sadrže sve esencijalne aminokiseline. Omjer esencijalnih aminokiselina u algama veći je od referentnog proteina prema prijedlogu FAO/WHO/UNU, osim za triptofan kojega ima najmanje u obje analizirane alge. Alge sadrže veliku količinu vitamina B_1, B_2, B_6, niacina i β-karotena te joda kao najvažnijeg sastojka alga. Utvrđena je velika količina minerala i neznatna količina teških metala.