

## CHEMICAL COMPOSITION AND EVALUATION OF PROTEIN QUALITY BY AMINO ACID SCORE METHOD OF EDIBLE BROWN MARINE ALGAE ARAME (*EISENIA BICYCLIS*) AND HIJIKI (*HIJIKIA FUSIFORME*)

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Crude proteins and their amino acid composition, fats, carbohydrates, cellulose, ashes, nucleic acids and minerals were determined in two edible and commercially available brown marine algae (*Phaeophyceae*), Arame (*Eisenia bicyclis*) and Hijiki (*Hijikia fusiforme*). The essential amino acid ratios for five key essential amino acids as well as the amino acid score based on the first limiting amino acid, Lys-Met-Cys score, Lys-Met-Cys-Trp score and Lys-Met-Cys-Trp-Thr score were calculated. The results have shown:

- rather high contents of proteins, containing all essential amino acids
- high amino acid ratios which are nearly as high as the value suggested by FAO/WHO/UNU pattern or higher
- the first limiting amino acid in both analysed algae is tryptophane
- very low contents of fats and nucleic acids
- high contents of cellulose and other carbohydrates
- large quantities of minerals and very low amounts of heavy metals.

**Keywords:** Arame, Hijiki, chemical composition, protein quality, amino acid scoring

*Eisenia bicyclis* and *Hijikia fusiforme*, commercially named Arame and Hijiki, respectively, are marine macroalgae classified as brown algae or *Phaeophyceae*. In Japan and the Far East these marine algae are regularly utilised in human alimentation since ancient times, while in European countries their use is only occasional (LAHAYE, 1991). With the spreading of the so called alternative diet like macrobiotic way of nutrition, the edible marine algae although in small quantities are consumed daily.

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Generally the marine algae are a good source of carbohydrates (FLEURY & LAHAYE, 1991), proteins, vitamins and minerals (FUJIWARA-ARASAKI et al., 1984). The algal carbohydrates: carrageenan, agar, alginate, cellulose, laminarans, fucans are undigestible by man and therefore considered dietary fibres (FLEURY & LAHAYE, 1991). Furthermore, due to their polysaccharidic structure, brown algae are particularly suitable for binding metallic ions, so lead and cadmium can be easily removed from dilute solutions by algal biomass (VOLESKY & HOLAN, 1995).

The algal proteins contain all essential amino acids, and the vitamins B-complex,  $\beta$ -carotene and vitamin C are determined.

The contents of iodine, calcium and iron are claimed to be greater than in vegetables and fruits used in traditional western diet (FUJIWARA-ARASAKI, 1984).

Marine algae are the source of safe and effective natural antioxidants (CAHYANA et al., 1992). As for some medical applications, the marine algae can develop antibiotic activity against multi-antibiotic resistant bacteria (MAHASNEH et al., 1995), and play a protective role against carcinogens (OKAI et al., 1997).

The objective of this work was to evaluate Arame and Hijiki as natural and useful food supplements in our western staple diet.

## 1. Materials and methods

Arame (*Eisenia bicyclis*) and Hijiki (*Hijikia fusiformis*) imported from Japan, were bought dry in a local health store. The samples were pulverised and the chemical composition (crude proteins ( $N \times 6.5$ ), fat, cellulose, ash and iodine) was determined according to A.O.A.C. method (1990). Minerals were determined by atomic absorption spectrophotometer Perkin Elmer 3030 B according to A.O.A.C. (1990). Phosphorous was determined by spectrophotometric method according to A.O.A.C. method (1990). Nucleic acids were determined by spectrophotometric method (RUT, 1973).

### 1.1 Amino acids analyses

**1.1.1. Chemicals.** DABS-Cl was purchased from Fluka (Buchs, Switzerland). A standard calibration mixture of amino acids of the highest grade and  $6 \text{ mol l}^{-1}$  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 through a  $0.22 \mu\text{m}$  Millipore filter (Millipore Corp., Milford, MA).

*1.1.2. Preparation of DABS-derivatives.* DABS-Cl solution ( $4 \text{ nmol } \mu\text{l}^{-1}$  acetonitrile) was prepared according to the method of CHANG and co-workers (1983) with slight modifications (STOCCHI et al., 1985; STOCCHI et al., 1989). Dried samples were resuspended in  $50 \text{ } \mu\text{l}$  of  $0.2 \text{ mol l}^{-1}$  sodium hydrogen carbonate, pH 9.0 and treated with  $100 \text{ } \mu\text{l}$  of DABS-Cl solution ( $4 \text{ nmol } \mu\text{l}^{-1}$  acetonitrile). The mixture was left at  $70^\circ\text{C}$  for 10 min and then dried under vacuum. The residue was redissolved in a proper volume of 70% (v/v) ethanol, and  $20 \text{ } \mu\text{l}$  of this volume was used for amino acids determination.

*1.1.3. Liquid phase hydrolysis with HCl* ( $c=6 \text{ mol l}^{-1}$ ). The pyrex hydrolysis tubes ( $10 \text{ cm} \times 7 \text{ mm}$  i.d.) for high sensitivity amino acids analysis were freed from amino acid contaminations by heating at  $600^\circ\text{C}$  overnight. Hydrolysis was carried out as follows:  $10 \text{ mg}$  of each dried and powdered sample was placed into a pyrex tube, treated with  $1 \text{ ml}$  of  $6 \text{ mol l}^{-1}$  hydrochloric acid (constant boiling, sequanal grade), sealed under vacuum and left at  $110^\circ\text{C}$  for 22 h.

Twenty  $\mu\text{l}$  of the hydrolysed sample were pipetted into a test tube, dried under vacuum and derivatized (STOCCHI et al., 1989).

*1.1.4. Alkaline hydrolysis with NaOH.* Alkaline hydrolysis, using  $4.2 \text{ mol l}^{-1}$  NaOH, was performed to check the recovery of tryptophane in protein samples with high sugar concentration. The hydrolysis was performed according to the method of HUGLI and MOORE (1972).

*1.1.5. 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  $20 \text{ } \mu\text{l}$  injection loop, and a Model 166 variable wavelength UV-visible range detector, equipped with a  $12 \text{ } \mu\text{l}$  flow cell. Integration of peak areas was obtained by means of the Gold software. Separation of DABS-amino acids was performed using a  $3 \text{ } \mu\text{m}$  Supelcosil LC-18 T column ( $15 \text{ cm} \times 4.6 \text{ mm}$  i.d.) protected with a  $5 \text{ } \mu\text{m}$  Supelcosil LC-18 T guard column ( $2 \text{ cm} \times 4.6 \text{ mm}$  i.d.) obtained from Supelco (Supelco, Bellefonte, PA). Solvent A was  $25 \text{ mM}$  potassium dihydrogen phosphate buffer, pH 7.05 and solvent B was 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  $1.5 \text{ ml min}^{-1}$  and detection was performed at  $436 \text{ nm}$ .

## 2. Results and discussion

Results are shown in Tables 1–5.

The protein contents in analysed algae are 11.1 g/100 g d.w. for Arame and 7.6 g/100 g d.w. for Hijiki (Table 1). The amino acid analysis revealed the presence of all amino acids including all essential amino acids (Table 2). The amount of amino acids in Arame and Hijiki was found to be 9.2% of dry weight and 6.7% of dry weight, respectively. It is interesting that in both analysed algae valine is present in the highest amount i.e. 14.0% of total amino acid content, followed by glutamic acid with 13.0% in Arame and 11.0% in Hijiki (Table 2). The presence of essential amino acids is 49.3% in Arame and 45.2% in Hijiki of the total amino acid content. Tryptophane, methionine, cystine, lysine and threonine are five key essential amino acids which are likely to be deficient in mixed human diet (SARWAR & MC DONOUGH, 1990). In both analysed algae tryptophane was found in the lowest amount (Tables 2, 3) and represents the first limiting amino acid with amino acid scores of 27 for Arame and 18 for Hijiki (Table 4). In Hijiki cystine was not determined, but the presence of a fairly high content of methionine with an amino acid ratio of 21 is about as high as the contents of sulfur containing amino acids (methionine + cystine) in FAO/WHO/UNU reference protein (FAO/WHO/UNU, 1985) (Table 3). In Arame and Hijiki the amino acid ratio of lysine is about 20% lower compared with the reference protein, while threonine in both analysed algae exceeds the recommended value (Table 3). In macrobiotic diet Arame and Hijiki are consumed with rice (LEDVINKA, 1986). As the first limiting amino acid in rice is lysine followed by threonine (HOSENEY, 1994), the amino acid content of Arame and Hijiki could be considered supplementary. Rather high values of amino acid scores Lys-Met-Cys, Lys-Met-Cys-Trp and Lys-Met-Cys-Trp-Thr (Table 4) would indicate a good protein quality. However, these data should be corrected for the protein digestibility in vivo (SARWAR et al., 1989).

The fat content (Table 1) in analysed algae are very low (0.90 g/100 g d.w. in Arame and 0.70 g/100 g d.w. in Hijiki). According to the literature the algae are richer in unsaturated fatty acids than the land plants. In *Phaeophyceae* the unsaturated fatty acids are mainly oleic and arachidonic acids (HARWOOD et al., 1988).

Table 1

*Chemical composition of marine algae Arame (Eisenia bicyclis)  
and Hijiki (Hijikia fusiforme) in g/100 g d. w.*

Chemical composition	Arame		Hijiki	
	$\bar{x}$	$\pm s$	$\bar{x}$	$\pm s$
Proteins	11.10	0.75	7.50	0.20
Fats	0.90	0.05	0.70	0.02
Cellulose	21.50	1.20	25.50	1.40
Ash	12.00	0.80	17.10	0.90
Carbohydrates	54.50	3.60	49.20	2.50
Nucleic acids	0.61	0.08	0.54	0.07

$\bar{x}$ : means of 3 determinations

$\pm s$ : standard deviation of 3 determinations

Table 2

*Amino-acid composition of marine algae Arame (Eisenia bicyclis)  
and Hijiki (Hijikia fusiforme) in mg/g d. w.*

Amino acid	Arame		Hijiki	
	$\bar{x}$	$\pm s$	$\bar{x}$	$\pm s$
Aspartic acid	7.18	0.36	6.86	0.34
Glutamic acid	11.95	0.52	7.48	0.30
Serine	3.51	0.25	2.63	0.26
•Threonine	6.24	0.59	3.34	0.31
Glycine	5.26	0.25	4.20	0.20
Alanine	8.88	0.42	4.96	0.23
Arginine	2.23	0.15	1.75	0.08
Proline	3.37	0.34	2.90	0.28
•Valine	12.86	1.15	9.47	0.85
•Methionine	1.44	0.13	1.54	0.14
•Isoleucine	4.35	0.37	4.48	0.40
•Leucine	6.99	0.34	5.99	0.26
•Phenylalanine	4.40	0.20	3.95	0.19
•Lysine	5.02	0.25	3.61	0.18
•Histidine	3.57	0.18	1.92	0.09
Tyrosine	2.33	0.12	1.99	0.09
Cystine	1.84	0.07	N.D.	N.D.
•Tryptophane	0.35	0.028	0.17	0.015
Total	91.76	5.718	67.24	4.215

•: Essential amino-acids

$\bar{x}$ : means of 3 determinations

$\pm s$ : standard deviation of 3 determinations

Table 3

*The essential amino-acid ratios of Arame (Eisenia bicyclis) and Hijiki (Hijikia fusiforme) compared with FAO/WHO/UNU suggested pattern of amino acid requirements in mg/g of protein*

Amino acid	Arame	Hijiki	FAO/WHO/UNU (1985) reference protein
HIS	32	25	19
ILE	39	60	28
LEU	63	80	66
LYS	45	48	58
MET+CYS	29	21	25
PHE+TYR	61	80	63
THR	56	45	34
TRP	3	2	11
VAL	116	126	35
Total	444	487	339
MET	13	21	
CYS	16	N.D.	
TYR	21	27	
PHE	40	53	

The amount of carbohydrates is high: 54.5 g/100 g d.w. for Arame and 49.8 g/100 g d.w. for Hijiki (Table 1). In brown algae carbohydrates represent the dietary fibres and consist of laminarans, alginates, fucans (LAHAYE, 1991). As the consumption of low-fibre diets is considered a common etiological factor in "civilisation" diseases like digestive problems, metabolic and cardiovascular diseases (TROWELL, 1976), the consumption of algae with other food rich in dietary fibres is obviously an important factor in self-protection of health and improvement of life. Furthermore, in *Phaeophyceae* the major polysaccharidic component is alginate which is known to have a particular affinity for binding bivalent heavy metals, and form slightly soluble salts, which are excreted by faeces (CONCON, 1988).

The contents of nucleic acids (Table 1) are low. If consumed by man the nucleic acids are metabolised to uric acid, which although mainly excreted by kidney can enter blood circulation. Too high amounts of uric acid in blood circulation could increase the risk to some diseases like gout, arthritis and kidney disorders (LEHNINGER, 1982), so the daily intake from unconventional sources like marine algae should not exceed 2.0 g of nucleic acids in addition to the usual diet (EDOZIEN, et al., 1970). It means that the intake of about 300 g d.w. of Arame and 440 g d.w. of Hijiki should be permitted.

Table 4  
*Amino acid scores (%) for Arame (Eisenia bicyclis)*  
*and Hijiki (Hijikia fusiforme)*

Algae	Amino acid score	Lys-Met-Cys score	Lys-Met-Cys Trp score	Lys-Met-Cys Trp-Thr score
Arame	27	96	73	96
Hijiki	18	83	62	79

According to the results in (Table 5) it can be seen that Arame and Hijiki have high contents of calcium, magnesium and phosphorus. The recommended daily dose for adults up to 25 years old of calcium is 800 mg, of magnesium 350 mg and of phosphorous 800 mg (WHITNEY & ROLFES, 1996). The presence of high calcium contents can represent a very good supplement in a diet which is often deficient in calcium for insufficient consumption of milk.

Table 5  
*Mineral composition of marine algae Arame (Eisenia bicyclis) and Hijiki (Hijikia fusiforme) in mg/100 g d.w.*

Minerals	Arame		Hijiki	
	$\bar{x}$	$\pm s$	$\bar{x}$	$\pm s$
Fe	2.69	0.06	4.35	0.09
Zn	1.32	0.04	0.891	0.021
Ca	946	20	1156	29
P	490	15	625	10
Mg	410	12	634	19
I	245	7	30	3
Cu	0.989	0.025	0.946	0.020
Mn	0.242	0.015	0.628	0.040
Cd	0.027	0.006	0.018	0.005
Ni	0.058	0.01	0.035	0.007
Cr	<0.005		<0.005	
Pb	0.046	0.012	0.095	0.038
Hg	0.025	0.008	0.027	0.005

$\bar{x}$ : Means of 3 determinations

$\pm s$ : Standard deviation of 3 determinations

Arame and Hijiki contain 245 and 30 mg/100 g d.w. of iodine, respectively (Table 5). As the essential trace element iodine is an integral part of two hormones released by the thyroid gland, and its recommended daily dose for adults is 150 µg (0.150 mg) (WHITNEY & ROLFES, 1996). The daily requirement of iodine would therefore be satisfied with 61 mg d.w. of Arame and 500 mg d.w. of Hijiki. The deficiency of iodine as well as its excessive accumulation in the tissue can cause serious health impairments. The toxic dose of iodine for adults is over 2000 µg (2 mg) per day (WHITNEY & ROLFES, 1996), meaning that the intake of 816 mg d.w. of Arame and 6.67 g of Hijiki per day would be allowed.

The same daily intake of Arame and Hijiki would supply 8 and 77 mg of calcium, 4 and 42 mg of phosphorous, respectively while the contribution of magnesium would be 3 and 42 mg, respectively. The allowed daily amount of Hijiki would therefore supply as much as 10% of daily dose in calcium, 5% in phosphorous and 12% in magnesium.

Three heavy metals mercury, lead and cadmium are the most toxic for the man (WHITNEY & ROLFES, 1996). The determined quantities of mercury (Table 5) is much inferior to the tolerable weekly intake, of 0.3 mg total mercury per person (CONCON, 1988) i.e. cca 0.04 mg daily, established by FAO/WHO Expert Committee in 1972. Allowed daily cadmium intake in humans established by FAO/WHO Expert Committee is 50–150 µg (0.05–0.15 mg), meaning that daily intake of 200 g d.w. of analysed algae hardly reaches the critical quantity of cadmium. In 1971 WHO suggested a limit for lead of 100 µg l<sup>-1</sup> of drinking water i.e. 250 µg daily, if it is assumed that 2.5 l of water is consumed daily (CONCON, 1988). In Hijiki where the quantity of lead is found to be twice as high as in Arame, the daily intake of cca 300 g d.w. would be allowed.

### 3. Conclusions

- The proteins of analysed algae contain all essential amino acids with high values of amino acid ratios except for tryptophane, which represents the first limiting amino acid.
- The high contents of undigestible carbohydrates contribute to the total amount of dietary fibres consumed daily, representing an important factor of self-protection.
- The analysed algae, especially Arame, are the source of iodine, the quantity of which should limit algal daily intake.
- In combination with other food the analysed algae contribute to satisfy the recommended doses in calcium, magnesium and phosphorous.
- Considering the low amount of nucleic acids and heavy metals, the analysed algae consumed as food supplement can improve the nutritive value of our staple diet.



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