ELSEVIER

Food Chemistry 72 (2001) 11–17

Food Chemistry

www.elsevier.com/locate/foodchem

Nutritional evaluation of some subtropical red and green seaweeds Part II. In vitro protein digestibility and amino acid profiles of protein concentrates

K.H. Wong, Peter C.K. Cheung *

Department of Biology, The Chinese University of Hong Kong, Shatin, New Territories, Hong Kong

Received 15 February 2000; accepted 24 May 2000

Abstract

The nutritional values of seaweed protein concentrates (PCs) isolated from two red seaweeds (*Hypnea charoides* and *Hypnea japonica*) and one green seaweed (*Ulva lactuca*) were evaluated by determining their in vitro protein digestibility and amino acid profiles. Both protein extractability and in vitro protein digestibility of the red seaweed PCs (88.7–88.9%) were significantly ($P < 0.05$, ANOVA, Tukey-HSD) higher than those of green seaweed PCs (85.7%). The total amount of essential amino acids (EAAs) in the three seaweed PCs was high (36.2–40.2% of total amino acid content). All three seaweed PCs were rich in leucine, valine and threonine but lacked cystine. However, except for sulphur-containing amino acids and lysine, the levels of all EAAs were higher than those of the FAO WHO requirement pattern. Relationships between total phenolic content in seaweeds and protein extractability as well as those between total phenolic content in seaweed PCs and in vitro protein digestibility are examined.

Keywords: In vitro protein digestibility; Amino acid profile; Seaweed protein concentrates

1. Introduction

The production of plant protein concentrates (PCs) is of growing interest to the food industry because of the increasing utilization of plant proteins in food, especially in developing countries (Akintayo, Esuoso & Oshodi, 1998; Sanchez-Vioque, Clemente, Vioque, Bautista & Millan, 1999). The use of plant PCs in food as functional ingredients, either to improve the nutritional quality of the product or for economic reasons, is very common. For example, soybean PCs (Qi, Hettiarachchy & Kalapathy, 1997) have been widely used as foaming, emulsifying, water binding and viscosity-modifying agents in food. However, these applications in the food trade are almost limited to protein from legumes (Chau, Cheung & Wong, 1997; Qi et al.; Sanchez-Vioque et al.) and cereals (Jayaprakasha & Brueckner, 1999; Prakash, 1996), whereas other plant proteins are less used.

Seaweeds belonging to the Rhodophyta (e.g. *Porphyra*) and Chlorophyta (e.g. *Ulva*) contain substantial amount of proteins (10–47% DW) with potential for human and animal nutrition (e.g. as functional food and fish feed) (Fleurence, 1999). However, only a few studies have been undertaken on the quality of the seaweed proteins (Amano & Noda, 1990; Dam, Lee, Fry & Fox, 1986; Fleurence; Ito & Hori, 1989). Extraction of proteins from seaweed is difficult because of the occurrence of phenolic compounds (Ragan & Glombitza, 1986) and large amounts polyanionic cell wall mucilages (Fleurence, Le Coeur, Mabeau, Maurice & Landrein, 1995; Jordan & Vilter, 1991). Phenolic compounds can destroy native protein structures that are attached to them and, under oxidizing conditions, can couple covalently to them (Loomis & Battaile, 1966). Cell wall mucilages form highly viscous solutions, disturbing extraction and purification procedures for proteins (Fleurence et al.; Ochiai, Katsuragi & Hashimoto, 1987). However, after comparing with different classical and enzymatic procedures (e.g. using an aqueous polymer two-phase system, polysaccharidases, or Tris HCl buffer), Fleurence et al. concluded that the highest

* Corresponding author. Tel.: +852-26096144; fax: +852-26035646.

E-mail address: petercheung@cuhk.edu.hk (P.C.K. Cheung).

0308-8146/00/$ - see front matter
PII: S0308-8146(00)00176-X

yield of seaweed PCs could be obtained by the use of NaOH and 2-mercaptoethanol after an initial aqueous extraction.

Digestion of seaweed proteins by proteolytic enzymes such as pepsin, pancreatin, pronase, trypsin, chymotrypsin has been reported (Fleurence, Chenard & Lucon, 1999; Fujiwara-Arasaki, Mino & Kuroda, 1984; Indegaard & Minsaas, 1991). However, the in vitro protein digestibility reported in these studies was only based on single enzyme digestion of seaweed protein. Multi-proteolytic enzyme system data, which are more similar to the actual digestion environment in vivo, on seaweed proteins are limited.

The objective of this study was to evaluate the nutritional value of the PCs isolated from three subtropical seaweeds, *H. charoides*, *H. japonica* and *U. lactuca* by determining their in vitro protein digestibility and amino acid profiles, in order to investigate their potential as new plant protein sources. Besides, the relationships between total phenolic content in seaweeds and protein extractability as well as total phenolic content in seaweed PCs and in vitro protein digestibility are examined.

2. Materials and methods

2.1. Sample preparation

All samples of seaweed were collected from A Ma Wan (AMW) and Lung Lok Shui (LLS) at Tung Ping Chau, in the northeast of Hong Kong. *H. charoides* and *H. japonica* (red seaweeds) were collected from both LLS and AMW in December 1997, while *U. lactuca* (green seaweed) was only collected from AMW in December 1997. Fresh plants were thoroughly washed with distilled water and their holdfasts and epiphytes were removed. All cleaned seaweeds were dried in a 60°C air oven for 15 h. All samples were dried to constant weight. The dried samples were pulverized by using a cyclotech mill (Tecator, Hoganas, Sweden) to pass through a screen with an aperture of 0.5 mm. The milled seaweed samples were then stored in air-tight plastic bags in desiccators at room temperature (25°C) prior to seaweed PCs extraction.

2.2. Extraction of seaweed protein concentrates

Red and green seaweed PCs were extracted using the method described by Fleurence et al. (1995) with slight modifications. In brief, 150 g of seaweed powder were suspended in de-ionized water (1: 20 w/v) to induce cell lysis by osmotic shock that facilitated subsequent protein extraction. Then the suspension was gently stirred overnight at 35°C, which was found to be the optimal temperature for seaweed protein solubility (Dua, Kaur & Ahluwalia, 1993). After incubation, the suspension was centrifuged at 10,000×*g* and 4°C for 20 min. The supernatant was collected and the pellet was re-suspended in de-ionized water in the presence of 0.5% (v/v) 2-mercaptoethanol (Venkataraman & Shivashankar, 1979). Then the pH of the mixture was adjusted to 12 with 1 M NaOH. The mixture was gently stirred at room temperature for 2 h before centrifugation under the same conditions as above. The second supernatant was collected and combined with the previous supernatant. The combined supernatant was stirred at 0–4°C and its pH was adjusted to 7 before precipitation with solid ammonium sulphate. The extraction procedure mentioned above was repeated five times on the residue.

2.3. Recovery of seaweed protein concentrates

Seaweed PCs were precipitated from the supernatant by slowly adding solid ammonium sulphate with stirring until a 85% saturation (60 g/100 ml) was reached (Rosenberg, 1996). Then the mixture was allowed to stand for 30 min before centrifugation under the same conditions as above. The pellet (PCs) obtained was dialyzed against distilled water until the total dissolved solutes (TDS) (mg/l) of dialysate, measured by its conductivity, was similar to that of the distilled water. Then the retentates, containing the seaweed PCs, were freeze-dried, ground to powder and stored in air-tight bags in desiccators before evaluation of their protein quality was preformed.

2.4. Crude protein content analysis

The percentages of crude protein of the red and green seaweeds as well as their PCs were calculated by multiplying the nitrogen content, which was determined by a CHNS/O analyzer (Perkin Elmer 2400, Connecticut, USA) by a factor of 6.25.

2.5. Extraction of total phenolic compounds

The total phenolic contents in the red and green seaweeds, as well as their PCs, were extracted according to the method described by Velioglu, Mazza and Oomah (1998). For *H. charoides*, *H. japonica* and *U. lactuca* (including their PCs), the optimal extraction conditions were 80% acetone for 6 h to achieve the optimal yield of total phenolic compounds (unpublished data). In brief, 100 mg of *H. charoides*, *H. japonica* and *U. lactuca* (as well as their PCs) were separately placed in test tubes and extracted with 10 ml of 80% acetone for 6 h. Each solvent system also contained 1% hydrochloric acid and all test tubes were incubated at room temperature (25°C) on an orbital shaker set at 200 rpm.

2.6. Determination of total phenolic contents

The total phenolic contents in red and green seaweeds, as well as their PCs, were analyzed in triplicate by the

standard Folin–Ciocalteu method (Singleton & Rossi, 1965) with slight modifications. One hundred microlitres of sample extract were placed in a test tube to which 0.9 ml de-ionized water, as well as 0.5 ml Folin–Ciocalteu reagent (catalogue no F9252, Sigma Chemical Co., St. Louis, MO, USA), were added. After 1 min, 1.5 ml 20% Na_2CO_3 were added and vortex mixed and the reaction mixtures allowed to stand for 60 min in darkness. The total phenolic contents were determined colorimetrically at 750 nm using a spectrophotometer (Spectronic Genesys G5, NY, USA). Gallic acid (catalogue no G7384, Sigma) was used as a standard and total phenolic compounds in the seaweed samples or PCs were expressed as milligrams of gallic acid equivalents (GAE) per gram of seaweed or GAE per gram of PCs on a dry weight basis (Julkunen-Tiitto, 1985).

2.7. In vitro protein digestibility of seaweed PCs

The in vitro protein digestibility was determined by the multi-enzyme method of Hsu, Vavak, Satterlee and Miller (1977). The enzymes used were porcine pancreatic trypsin (activity: 15200 units/mg of protein, T0134, Sigma), bovine pancreatic chymotrypsin (activity: 54 units/mg of protein, C4129, Sigma) and porcine intestinal peptidase (activity: 102 units/g of solid, P7500, Sigma). A 5 ml quantity of enzyme solution (23100 units of trypsin, 186 units of chymotrypsin and 0.052 units of peptidase/ml) was prepared at pH 8 and 37°C. A 50 ml quantity of protein suspension, of concentration 6.25 mg of protein/ml of distilled water, was also prepared for each seaweed PCs at the same pH and temperature. The 5 ml enzyme solution and 50 ml protein substrates were mixed. The pH change of the mixture after exactly 10 min was measured and the percentage of in vitro protein digestibility (Y) was computed using the equation $Y = 210.464 – 18.10X$, where X is the pH change after 10 min. Sodium caseinate (catalogue no. C8654, Sigma) was used as control and the in vitro protein digestibility of seaweed PCs was expressed as a relative percentage of that of the sodium caseinate normalized at 100% (FAO/WHO, 1991).

2.8. Amino acid analysis of seaweed PCs

Two milligrams of seaweed PCs were hydrolyzed with 0.5 ml 6 M HCl (catalogue no. H0636, Sigma) in a sealed ampoule containing 8 μl phenol (for protection of tyrosine) and 0.25 μl mol norleucine (catalogue no. N8513, Sigma) as an internal standard for 24 h at 110°C under vacuum. The acid hydrolysate was evaporated to dryness using a Speedvac concentrator (Savant Instrument, Farmingdale, NY) and the dry residue was redissolved in 0.5 ml of citrate buffer (Beckman A303084, CA). The sample was filtered through a 0.45 μm nylon filter before being analyzed with an automated amino acid analyzer (Beckman 6300, CA). Sulphur-containing amino acids, cystine and methionine, were determined after a pre-hydrolysis oxidation with performic acid (Gehrke, Wall, Absheer, Kaiser & Zumwalt, 1985). The contents of different amino acids recovered are presented as mg g^{-1} protein and are compared with the FAO/WHO (1991) reference pattern. The essential amino acid (EAA) score was calculated by the method of FAO/WHO as shown below:

$$\text{Essential amino acid score} = \frac{\text{mg of EAA in 1g of test protein}}{\text{mg of EAA in 1g of egg protein}} \times 100$$

2.9. Statistical analysis

All analyses were performed in triplicate. Except for the amino acid profiles, all data are presented as mean values ±S.D., the mean values being analyzed by one-way ANOVA and Tukey-HSD at $P < 0.05$ (Wilkinson, 1988) to detect significant differences among groups. The assumptions of the parametric statistics were satisfied.

3. Results and discussion

3.1. Protein extractability

Table 1 shows that, when subjected to oven-drying, the crude protein content (7.11–19.4% DW) of *Hypea* and *Ulva* species lay within the range for red and green seaweeds (10–47% DW) reported by Fleurence (1999). Also, the crude protein content of the *U. lactuca* (7.11% DW) was significantly ($P < 0.05$, ANOVA, Tukey-HSD) lower than that of the two red seaweeds (*H. charoides* and *H. japonica*) (18.1–19.4% DW).

Table 1 also shows that the % N, % protein, sample dry weight, amount of protein extracted and % yield of the red seaweed PCs were significantly ($P < 0.05$, ANOVA, Tukey-HSD) higher than those of PCs from the green seaweed. This implied that the protein extractability of red seaweeds was higher than that of green seaweed. Also, the % N of the three seaweed PCs (ranged from 12.2 to 13.6%) agreed with the results observed for other red (*Porphyra tenera* and *Grateloupia turuturu*) and green (*Ulva pertusa* and *Codium fragile*) seaweed PCs, which ranged from 13.2 to 15.8% (Arasaki & Mino, 1973; Fujiwara-Arasaki et al., 1984).

The presence of phenolic compounds in seaweeds has been known for a long time (Fujimoto, Ohmura & Kaneda, 1985; Pedersen, 1984; Ragan & Glombitza, 1986). In this study, the total phenolic content of the red and green seaweeds ranged from 8.48 to 8.99 mg/g DW. Furthermore, the total phenolic content in the red

Table 1
Total phenolic and total crude protein contents in *Hypnea charoides*, *Hypnea japonica* and *Ulva lactuca* as well as % nitrogen, % protein, sample dry weight, amount of protein extracted and % yield of their protein concentrates (PCs)[a]

Seaweeds	Total phenolic content, GAE in seaweeds (mg/g DW[b])	Total crude protein content in 150 g seaweeds (g)	% Nitrogen in PCs (%)	% Protein in PCs (% N×6.25)	Sample dry weight of PCs (g)	Amount of protein extracted in PCs[c] (g)	% Yield of PCs[d] (%)
H. charoides	8.44±0.53a	27.2±0.44a	13.3±0.15a	83.1±0.94a	15.2±0.20a	12.6±0.17a	46.3±0.61a
H. japonica	8.48±0.07a	29.1±0.50a	13.6±0.06a	85.0±0.38a	15.5±1.26a	13.2±1.07a	45.4±0.23a
U. lactuca	8.99±0.19b	10.7±0.32b	12.2±0.12b	76.3±0.75b	5.11±0.05b	3.90±0.04b	36.4±0.35b

[a] Data are mean values of three determinations±S.D. Means in a whole column with different letters (a,b) are significantly different ($P < 0.05$, ANOVA, Tukey-HSD).
[b] DW = sample dry weight.
[c] Amount of protein extracted in PCs = % protein in PCs × sample dry weight of PCs.
[d] % Yield = amount of protein extracted in PCs/total crude protein in 150 g seaweeds ×100.

seaweeds was significantly ($P < 0.05$, ANOVA, Tukey-HSD) lower than that of the green seaweed (Table 1).

As mentioned earlier, extraction of seaweed PCs is difficult because of the presence of large amounts of anionic or neutral polysaccharides as well as phenolic compounds, especially in brown seaweeds (Fleurence et al., 1995; Jordan & Vilter, 1991; Ragan & Glombitza, 1986). Cell wall polysaccharides form highly viscous solutions, which disturb the extractions and purification procedures for proteins (Amano & Noda, 1990; Fleurence, 1999; Fleurence et al.; Jordan & Vilter). Phenolic compounds may reversibly complex with proteins by hydrogen bonding or irreversibly by oxidation to quinines, which combine with reactive groups of the protein molecules (Loomis & Battaile, 1966). Such chemical reactions of the phenolic compounds with proteins would also limit the efficiency of protein extraction.

In this study, a high negative correlation ($r = -0.99$) between % yield of PCs and total phenolic contents of the seaweed was also obtained, indicating that high total phenolic content in the seaweed samples might result in a lower % yield of seaweed PCs. Moreover, the % yield of the seaweed PCs (ranged from 36.4 to 46.3%) was considerably higher than that (7.00–20.0%) reported by Fuijiwara-Arasaki et al. (1984). Although the % yield of *U. lactuca* PCs (36.4%) was significantly ($P < 0.05$, ANOVA, Tukey-HSD) lower than that of two *Hypnea* PCs (45.4–46.3%), it was comparable to that of *U. rotundata* (36.1%) and *U. rigida* PCs (26.8%) obtained in the previous report (Fleurence et al., 1995).

3.2. Protein quality

3.2.1. Total phenolic contents and in vitro protein digestibility of seaweed PCs

According to Hurrell and Finot (1985), one major factor that influences protein digestibility is the presence of phenolic compounds. Oxidized phenolic compounds may react with amino acids and proteins, inhibiting the activity of proteolytic enzymes (Milic, Stojanovic, Vucurevic & Turcic, 1968). The ability of phenolic compounds to form insoluble complexes with protein interferes with the utilization of dietary proteins, thus lowering their nutritional value (Shahidi & Naczk, 1995). Similarly, in the present study, the negative correlation between the in vitro protein digestibility and the total phenolic content in the PCs was also high ($r = -1.00$), implying that the higher the total phenolic content of seaweed PCs, the lower is the in vitro protein digestibility. However, the in vitro protein digestibility of the *Hypnea* and *Ulva* PCs (ranged from 85.7 to 88.9%) (Table 2) was comparable to that of other red and green seaweed PCs from Korea (78.5%) (Ryu, Satterlee & Lee, 1982). Also, the in vitro protein digestibility of the red seaweed PCs was significantly ($P < 0.05$, ANOVA, Tukey-HSD) higher that of the green. Fleurence (1999) reported that the in vitro protein digestibility of seaweed proteins differed according to the species and seasonal variations of the content of anti-nutritional factors such as phenolic molecules and polysaccharides (Fleurence; Indegaard & Minsaas, 1991; Mabeau & Fleurence, 1993).

3.2.2. Amino acid composition

The amino acid profiles and the essential amino acid scores of the red and green seaweed PCs are presented in Table 3. The amino acids analyzed represented both the free and combined amino acids. The amount of essential amino acids of the seaweed PCs accounted for 36.2–40.2% of total amino acid content {[Level of total EAAs (mg/g of protein)/sum of all measured amino acids (mg/g protein)]×100%} which was comparable to that of the other red and green seaweed PCs reported in earlier work: 37.0–37.9% in *Porphyra tenera*, *Grateloupia turuturu*, *Ulva pertusa* and *Codium fragile* (Fujiwara-Arasaki et al., 1984); 37.1-42.0% in *Ulva lactuca* and *Gelidium amansii* (Ochiai et al., 1987) and 36.5–38.6% in *Ulva rigida* and *Ulva rotundata* (Fleurence et al., 1995). For essential amino acids, the three seaweed PCs were rich in leucine, valine and threonine, which also agreed with previous reports (Ochiai et al.; Fleurence et al.). Also, the limiting amino acids of the *Hypnea*

Table 2
Total phenolic contents and in vitro protein digestibility of *Hypnea charoides*, *Hypnea japnoica* and *Ulva lactuca* protein concentrates[a]

Seaweed PCs[b]	Total phenolic contents, GAE in PCs (mg/g DW[c])	In vitro protein digestibility (%)
H. charoides	16.9±1.00a	88.7±0.70a
H. japonica	16.3±0.03a	88.9±1.40a
U. lactuca	38.8±0.50b	85.7±1.90b

[a] Data are mean values of three determinations±S.D. Means in columns with different letters (a,b) are significantly different ($P < 0.05$, ANOVA, Tukey-HSD).
[b] PCs = protein concentrates.
[c] DW = sample dry weight.

and *Ulva* seaweed PCs were the sulphur-containing amino acids (EAA score ranged from 0.24 to 0.79) and lysine (EAA score ranged from 0.68 to 0.80). This observation was in accordance with the data of seaweed PCs isolated from *Ulva pertusa*, *Codium fragile*, *Porphyra tenera* and *Grateloupia turututu* (Arasaki & Mino, 1973; Fujiwara-Arasaki et al.). Except for the sulphur-containing amino acids (methionine and cystine) and lysine, the levels of all the EAAs were higher than those of the FAO/WHO requirement pattern (FAO/WHO, 1991) (Table 3). Furthermore, no cystine was detected in any of the seaweed PCs, which is consistent with results reported by several authors (Arasaki & Mino; Fleurence et al.; Fleurence et al., 1999).

All seaweed PCs exhibited similar non-essential amino acid patterns in which aspartic and glutamic acids were the predominant types (25.6–31.0% of total AA). This observation was in accordance with previous reports on other red and green seaweed PCs: 24.0–35% in *Ulva amoricana* (Fleurence et al., 1999); 26.0–31.5% in *Ulva rigida* and *Ulva rotundata* (Fleurence et al., 1995) and 21.8–25.6% in *Porphyra Suborbiculata*, *Enteromopha linza* and *Ulva pertusa* (Woo, Ryu & Lee, 1979). Moreover, the seaweed PCs were rich in glycine and alanine but poor in histidine, which was also consistent with the results of seaweed PCs such as *Ulva pertusa*, *Codium fragile*, *Porphyra tenera*, *Grateloupia turututu* (Arasaki & Mino, 1973), *Ulva rigida*, *Ulva rotundata* (Fleurence et al., 1995), *Ulva lactuca* and *Gelidium amansii* (Ochiai et al., 1987).

In this study, there were some pronounced differences between the amino acid profiles of red and green seaweed PCs. According to Arasaki and Mino (1973), higher levels of proline were obtained in red seaweed PCs (*Porphyra tenera* and *Grateloupia turututu*) (6.42–6.59% of total AA) than in green seaweed PCs (*Ulva pertusa* and *Codium fragile*) (4.65–4.83% of total AA). However, in this study the red seaweed PCs (*H. charoides* and *H. japonica*) were characterized by their relatively higher arginine level (10.4–10.6% of total AA; green seaweed PCs (*U. lactuca*): only 5.00% of total AA). Similar results (red seaweed: 19.5–19.6% of total AA; green seaweed PCs: 15.6–17.8% of total AA) were

Table 3
Amino acid profiles (mg g^{-1} protein)[a] of the *Hypnea charoides*, *Hypnea japonica* and *Ulva lactuca* protein concentrates

Amino acids	*H. charoides*	*H. japonica*	*U. lactuca*	FAO/WHO (1991) requirement pattern
Aspartic acid	163	159	139	
Threonine	48.3 (1.42)	49.0 (1.44)	62.0 (1.82)	34
Serine	46.8	50.4	62.8	
Glutamic acid	125	126	110	
Proline	35.3	38.0	45.7	
Glycine	55.2	53.3	65.3	
Alanine	60.6	60.7	96.7	
Valine	52.1 (1.49)	52.6 (1.50)	70.1 (2.00)	35
Methionine	16.2 (0.65)[b]	19.7 (0.79)[b]	6.12 (0.24)[b]	25[b]
Cystine	0.00	0.00	0.00	
Isoleucine	39.2 (1.40)	46.0 (1.64)	40.0 (1.43)	28
Leucine	69.8 (1.06)	68.0 (1.03)	72.6 (1.10)	66
Tyrosine	29.1 (1.13)[c]	34.3 (1.27)[c]	36.3 (1.48)[c]	63[c]
Phenylalanine	42.2	46.0	57.1	
Histidine	7.67	11.0	13.1	
Lysine	39.2 (0.68)	44.6 (0.77)	46.4 (0.80)	58
Arginine	98.1	100	48.6	
Tryptophan	ND[d]	ND	ND	11
Total EAA[e]	336	371	391	320
Total AA (g/100g PCs)	78.7	78.7	73.9	—

[a] Values are the average of three determinations. Figures in parentheses are the essential amino acids score.
[b] Cystine + methionine.
[c] Tyrosine + phenylalanine.
[d] Not determined.
[e] Total essential amino acids (mg/g protein) excludes tryptophan.

reported by Fujiwara-Arasaki et al. (1984). For green seaweed PCs (*U. lactuca*), a notably higher alanine level (9.95% of total AA) was obtained when compared with that of the red seaweed PCs (*Hypnea* species) (6.33–6.53% of total AA). This phenomenon was consistent with the PCs of other *Ulva* species (7.29–8.11% of total AA) (Fleurence et al., 1995). Furthermore, in this study, the total amino acid content (ranged from 73.9 to 78.7 g/100 g PCs) of each seaweed PCs was comparable to their corresponding % protein (76.3–85.0%) (Table 1). This indicated that the amount of non-protein nitrogenous materials in the three seaweed PCs were insignificant.

4. Conclusions

With respect to the relatively higher crude protein content, protein extractability (% N, % protein, amount of protein extracted and % yield) and protein quality (in vitro protein digestibility and amino acid profile) of their PCs, the two *Hypnea* seaweeds are more potent alternative plant protein sources for human and animal nutrition than the *U. lactuca*. Although the in vitro protein digestibility is an easier and more rapid technique, it is only an approximation of the true protein digestibility and not as accurate as the in vivo method. Therefore, biological evaluation using human and animal feeding studies would be required to establish the actual nutritional values of the seaweed PCs studied here, particularly the in vivo protein digestibility.

Acknowledgements

We acknowledge the technical assistance of Mr. C.C. Li. This project was funded by the Research Grants Council of the Hong Kong SAR Government.

References

Akintayo, E. T., Esuoso, K. O., & Oshodi, A. A. (1998). Emulsifying properties of some legume proteins. *International Journal of Food Science and Technology, 33*, 239–246.

Amano, H., & Noda, H. (1990). Proteins of protoplast from red alga *Porphyra yezoensis*. *Bulletin of the Japanese Society of Scientific Fisheries, 56*, 1859–1864.

Arasaki, T., & Mino, N. (1973). The alkali soluble proteins in marine algae. *Journal of the Japanese Society of Food and Nutrition, 26*, 129–133.

Chau, C. F., Cheung, P. C. K., & Wong, Y. S. (1997). Functional properties of protein concentrates from three Chinese indigenous legume seeds. *Journal of Agricultural and Food Chemistry, 45*, 2500–2503.

Dam, R., Lee, S., Fry, P. C., & Fox, H. (1986). Utilization of algae as a protein source for humans. *Journal of Nutrition, 65*, 376–382.

Dua, S., Kaur, M., & Ahluwalia, A. S. (1993). Functional properties of two pollutant grown green algae. *Journal of Food Science and Technology, 30*, 25–28.

FAO/WHO. (1991). *Protein quality evaluation. Report of joint FAO/WHO expert consultation*. Rome, Italy: Food and Agriculture Organization of United Nation.

Fleurence, J. (1999). Seaweed proteins: biochemical, nutritional aspects and potential uses. *Trends in Food Science and Technology, 10*, 25–28.

Fleurence, J., Chenard, E., & Lucon, M. (1999). Determination of the nutritional value of proteins obtained from *Ulva armoricana*. *Journal of Applied Phycology, 11*, 231–239.

Fleurence, J., Le Coeur, C., Mabeau, S., Maurice, M., & Landrein, A. (1995). Comparison of different extractive procedures for proteins from the edible seaweeds *Ulva rigida* and *Ulva rotundata*. *Journal of Applied Phycology, 7*, 577–582.

Fujimoto, K., Ohmura, H., & Kaneda, T. (1985). Screening for antioxygenic compounds in marine algae and bromophenols as effective principles in a red alga *Polysiphonia ulceolate*. *Bulletin of the Japanese Society Scientific Fisheries, 51*, 1139–1143.

Fujiwara-Arasaki, T., Mino, N., & Kuroda, M. (1984). The protein value in human nutrition of edible marine algae in Japan. *Hydrobiologia, 116/117*, 513–516.

Gehrke, C. W., Wall, L. L., Absheer, J. S., Kaiser, F. E., & Zumwalt, R. W. (1985). Sample preparation for chromatography of amino acids: acids hydrolysis of proteins. *Journal of the Association of Official Analytical Chemists, 68*, 811–821.

Hsu, H. W., Vavak, D. L., Satterlee, L. D., & Miller, G. A. (1977). A multi-enzyme technique for estimating protein digestibility. *Journal of Food Science, 42*, 1269–1273.

Hurrell, R. F., & Finot, P. A. (1985). Effect of food processing on protein digestibility and amino acids availability. In J. W. Finely, & D. T. Hopkins, *Digestibility and amino acids availability in cereals and oilseeds* (pp. 233–246). St Paul, MN: American Association of Cereals Chemists.

Indegaard, M., & Minsaas, J. (1991). Animal and human nutrition. In G. Guiry, & G. J. Blundens, *Seaweed resources in Europe. Uses and potential* (pp. 21–64). New York: Wiley & Sons.

Ito, K., & Hori, K. (1989). Seaweed: chemical composition and potential uses. *Food Review International, 5*, 101–144.

Jayaprakasha, H. M., & Brueckner, H. (1999). Whey protein concentrate: a potential functional ingredient for food industry. *Journal of Food Science and Technology, 36*, 189–204.

Jordan, P., & Vilter, H. (1991). Extraction of proteins from material rich in anionic mucilages: partition and fractionation of vanadate-dependent bromoperoxidases from the brown algae *Laminaria digitata* and *L. saccharina* in aqueous polymer two-phase system. *Biochemical and Biophysical Acta, 1073*, 98–106.

Julkunen-Tiitto, R. (1985). Phenolic constituents in leaves of northern willows: methods for the analysis of certain phenolics. *Journal of Agricultural and Food Chemistry, 33*, 213–217.

Loomis, W. D., & Battaile, J. (1966). Plant phenolic compounds and isolation of plant enzymes. *Phytochemistry, 5*, 423–438.

Mabeau, S., & Fleurence, J. (1993). Seaweed in food products: biochemical and nutritional aspects. *Trends in Food Science and Technology, 4*, 103–107.

Milic, B., Stojanovic, S., Vucurevic, N., & Turcic, M. (1968). Chlorogenic and quinic acids in sunflower meals. *Journal of Science and Food Agriculture, 19*, 108–112.

Ochiai, Y., Katsuragi, T., & Hashimoto, K. (1987). Proteins in three seaweeds: "Aosa" *Ulva lactuca*, "Arame" *Eisenia bicyclis*, and "Makusa" *Gelidium amansii*. *Bulletin of the Japanese Society of Scientific Fisheries, 53*, 1051–1055.

Pedersen, A. (1984). Studies on phenol content and heavy metal uptake in fucoids. *Hydrobiologia, 116/117*, 498–504.

Prakash, J. (1996). Rice bran proteins: properties and food uses. *Critical Reviews in Food Science and Nutrition, 36*, 537–552.

Qi, M., Hettiarachchy, N. S., & Kalapathy, U. (1997). Solubility and emulsifying properties of soy protein isolates modified by pancreatin. *Journal of Food Science, 62*, 1110–1115.

Ragan M. A., & Glombitza, K. W. (1986). Phlorotannins, brown algal polyphenols. In F. E. Round, & D. J. Chapman, *Progress in phycological research* (Vol. 4, pp. 130–230). Bristol: Biopress Ltd.

Rosenberg, I. M. (1996). *Protein analysis and purification*. Boston: Birkhäuser.

Ryu, H. S., Satterlee, L. D., & Lee, K. H. (1982). Nitrogen conversion factors and in vitro protein digestibility of some seaweeds. *Bulletin of the Korean Fisheries Society, 15*, 263–270.

Sanchez-Vioque, R., Clemente, J., Vioque, J., Bautista, J., & Millan, F. (1999). Protein isolates from chickpea (*Cicer arietinum* L.): chemical composition, functional properties and protein characterization. *Food Chemistry, 64*, 237–243.

Shahidi, F., & Naczk, M. (1995). *Food phenolics: sources, chemistry, effects and applications*. Switzerland: Technonomic Publishing Company: Basel.

Singleton, V. L., & Rossi, J. A. (1965). Colorimetry of total phenolic with phosphomolybdic-phosphotungstic acid reagents. *American Journal of Enology and Viticulture, 16*, 144–158.

Velioglu, Y. S., Mazza, G., & Oomah, B. D. (1998). Antioxidant activity and total phenolics in selected fruits, vegetables and grain products. *Journal of Agricultural and Food Chemistry, 46*, 4113–4117.

Venkataraman, L. V., & Shivashankar, S. (1979). Studies on the extractability of proteins from the alga *Scenedesmus acutus*. *Arch. Hydrobiologia, 56*, 114–126.

Wilkinson, L. (1988). *SYSTAT: the system for statistics*. IL: Evanston.

Woo, S. I., Ryu, H. S., & Lee, K. H. (1979). Studies on the extraction of seaweed proteins 4. Precipitation conditions and nutritional evaluation of isolated seaweed proteins. *Bulletin of the Korean Fisheries Society, 12*, 225–234.