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Nutritional evaluation of protein concentrates isolated from two red seaweeds: *Hypnea charoides* and *Hypnea japonica* in growing rats

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Abstract

The nutritional values of protein concentrates (PCs) isolated from two subtropical red seaweeds, *Hypnea charoides* Lamouroux and *H. japonica* Tanaka, were evaluated in growing rats. The protein quality of the two seaweed PCs was determined by comparing the net protein ratio (NPR), true protein digestibility (TD), nitrogen balance (NB), biological value (BV), net protein utilization (NPU) and utilizable protein (UP) of the two seaweed PCs diet groups with those of the casein control group. There were no significant differences of NPR and BV in all diet groups. Although the values of TD (ranged from 90.5 to 90.6%), NB (ranged from 108 to 113 mg rat^{-1} day^{-1}), NPU (ranged from 80.1 to 81.3%) and UP (ranged from 80.1 to 81.3%) of these two PCs were significantly lower than those of the casein control, they were comparable to those of other common plant PCs. The growth performance of rats fed the two PCs diets was satisfactory and both PCs had no adverse effect on the weight of their major organs. Together with their good protein quality as mentioned above, the PCs from the two red seaweeds under study could be a potential alternative protein source for human nutrition.

Introduction

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

People in the Far East and Asia Pacific have a long tradition of consuming seaweeds as part of their diet. In the western countries, the principal uses of seaweeds are as sources of phycocolloids, thickening and gelling agents for various industrial applications including, foods (Darcy-Vrillon, 1993; Mabeau & Fleurence, 1993; Abbott, 1996). Recently in France, seaweeds have been approved for use as vegetable and condiments (Mabeau, 1989). Therefore, seaweeds are becoming a valuable vegetable (fresh or dried) and an important food ingredient in human diet nowadays, even in the western world.

The nutritional potential of seaweeds as food protein sources differs according to species (Fleurence et al., 1999). Seaweeds belonging to the Rhodophyta possess high levels of proteins (10–30% DW) (Darcy-Vrillon, 1993; Mabeau & Fleurence, 1993) comparable to those of edible land vegetables (around 20% DW, Dupin et al., 1992). In some red seaweeds, such as *Palmaria palmata* (L.) Kuntze (dulse) and *Porphyra tenera* Kjellman (nori), the protein contents are 35 and 47% DW, respectively (Morgan et al., 1980; Fujiwara-Arasaki et al., 1984). These levels are even comparable to that of the soybeans (35% DW). However, only a few studies have been undertaken on the quality of seaweed protein (Dam et al., 1986; Ito & Hori, 1989; Amano & Noda, 1990; Fleurence, 1999a) because of the difficulties of extraction and preparation

of seaweed PCs. The extraction of seaweed protein by classical procedures is hindered by the presence of large amounts of cell wall polysaccharides, such as the alginates of the brown seaweed or the carrageenans of some red seaweeds. The high content of neutral polysaccharides (e.g. xylans and cellulose) in some red and green seaweeds can also limit the protein accessibility (Fleurence, 1999b). These anionic and neutral polysaccharides are the main impediment during the extraction and purification of seaweed protein (Ochiai et al., 1987; Ito & Hori, 1989; Jordan & Vilter, 1991; Fleurence et al., 1995). Moreover, although *in vitro* seaweed protein degradation by proteolyic enzymes such as pepsin, pancreatin and pronase have been reported previously (Ryu et al., 1982; Fujiwara-Arasaki et al., 1984), the *in vivo* digestibility of seaweed proteins is not well documented (Mabeau & Fleurence, 1993; Fleurence, 1999a).

The extraction procedures for seaweed proteins described in the literature are mainly concerned with the extraction of specific seaweed enzymes such as proteases (Kadokami et al., 1990), peroxides (Sheffield et al., 1993) or carboxylases (Hilditch et al., 1991). In comparison, very little information about the extraction of the total protein fraction from seaweed is available (Fleurence et al., 1995). After comparing with different classical and enzymatic procedures (e.g. using aqueous polymer two-phase system, polysaccharidases, or Tris HCl buffer), Fleurence et al. (1995) reported that the highest yield of seaweed PCs was obtained by the use of NaOH and 2-mercaptoethanol after an initial aqueous extraction.

The inadequacy of bioassay techniques, including protein efficiency ratio (PER), for evaluating protein quality has been recognized (Pellett & Young, 1980; Madi, 1993). Biological indices like net protein ratio (NPR), nitrogen balance (NB), true protein digestibility (TD), biological value (BV), net protein utilization (NPU) as well as utilizable protein (UP), which are widely used in nutritional studies (Kalra & Jood, 1998; Wong & Cheung, 1998), are recommended by the FAO/WHO (1991) for evaluating protein quality. Besides, *in vivo* experiment using the rat balance method (McDonough et al., 1990) is more suitable to predict protein digestibility in humans (FAO/WHO, 1991).

In Hong Kong, the seaweed floras are fairly rich but they are relatively under-utilized (Hodgkiss & Lee, 1983). In general, most Hong Kong seaweeds are mainly used as animal feeds or fertilizers by the coastal villagers (Hodgkiss & Lee, 1983). *Hypnea charoides* Lamouroux and *H. japonica* Tanaka are two subtropical red seaweeds that are very abundant in Hong Kong. The aim of this study was to evaluate the nutritional value of the PCs isolated from these two subtropical red seaweeds in growing rats, as reflected by the computed biological indices mentioned above.

Materials and methods

Sample preparation

Samples of *H. charoides* and *H. japonica* were collected in December of 1997 from A Ma Wan and Lung Lok Shui at Tung Ping Chau, on the northeast of Hong Kong (114° 26′ E; 22° 33′ N). Fresh plants were thoroughly washed with distilled water and their holdfasts and epiphytes were removed. All cleaned seaweeds were then frozen at −70 °C for 24 h and then dried in a freeze-drier (Labconco, MO) for 5 days. All samples were dried to constant weight. The dried samples were pulverized by using a cyclotech mill (Tecator, Höganäs, 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.

Extraction of seaweed protein concentrates

Seaweed PCs were extracted with the method described by Fleurence et al. (1995) with slight modifications. In brief, seaweed powder was suspended in de-ionzed water (1:20 w/v) to induce cell lysis by osmotic shock in order to facilitate subsequent protein extraction. The suspension was then gently stirred overnight at 35 °C, which is the temperature found to be optimal for seaweed protein solubility (Dua et al., 1993). After incubation, the suspension was centrifuged at 10 000 × $\boldsymbol{g}$ 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). The mixture was then adjusted to pH 12 with 1 M NaOH and gently stirred at room temperature (25 °C) for 2 h before centrifugation at the same conditions mentioned above. The supernatant was collected and combined with the previous supernatant. The combined supernatants were stirred at 0–4 °C and adjusted to pH 7 before precipitation with solid ammonia sulphate. The extraction procedure mentioned above was repeated five times on the residue.

Precipitation of seaweed protein concentrates

Seaweed PCs were precipitated from the supernatant by slowly adding solid ammonia sulphate with stirring until a 85% saturation (60 g/100 ml) was reached (Rosenberg, 1996). The mixture was then allowed to stand for 30 min before centrifugation at the same conditions mentioned before. The pellet (PCs) obtained was dialysed against distilled water until the total dissolved solutes (TDS) (mg l^{-1}) of the dialysate, measured by its conductivity, was similar to that of the distilled water. The retentates containing seaweed PCs was then freeze-dried, ground to powder and stored in air-tight bags in desiccators before biological evaluation of their protein quality was performed. The percent crude protein of the seaweed PCs was calculated by multiplying percent nitrogen that was determined by a CHNS/O Analyzer (Perkin Elmer 2400, Connecticut, U.S.A.) with a factor of 6.25.

Diet preparation

The control and test diets were prepared according to the AIN-93G purified diet (Reeves et al., 1993) with slight modifications. All diets were formulated to contain identical levels of ingredients. All diets contained 10.0% of protein, as this level of protein is limiting for growth in weanling rat and any effect of seaweed PCs would be more likely to have a significant change on their protein utilization. The isoproteinous level was achieved by taking into account the purity of each protein source. Casein (90.0%, C7078, Sigma Chemical Co., St. Louis, MO) served as the sole protein source for the control group while the sole protein source in the test diets came from their corresponding seaweed PCs (*H. charoides* PCs: 83.1% and *H. japonica* PCs: 85.0%) (Wong & Cheung, 2001). This formulation allowed for constancy of gross energy density of each diet which was calculated by employing the factors: proteins = 4.00 Kcal g^{-1}; carbohydrates = 4.00 Kcal g^{-1}; fats = 9.00 Kcal g^{-1}. The formulations of the semi-purified test diets are shown in Table 1. A total of four diets were investigated: a casein-based control diet, a protein-free diet and two seaweed PCs diets. Each diet was made up in a single batch of about 1 kg and their moisture content was also determined with an infrared moisture analyzer (Mettler LJ 16, Greifensee, Switzerland) at 120 °C.

Rat bioassay

The experiment procedure was similar to the rat balance method described by McDonough et al. (1990). In brief, 16 male, weanling Sprague Dawley rats from the same colony with initial body weight of 50–70 g were obtained from the Laboratory Animal Service Center of The Chinese University of Hong Kong. Animals transported from breeding colony to test laboratory were weighed when received, and fed standardized laboratory rat chow for an acclimation period of 2 days. Four rats were then assigned randomly to each of the four experimental diet groups and were housed in individual metabolic cages kept under the conditions of 18–26 °C, 40–70% relative humidity and with 12 h light /dark cycle. All animals had free access to water and diets.

The rat balance method used in this study consisted of a 5-day preliminary period, during which the rats were allowed to adapt to the diets and experimental conditions, followed by a 5-day balance period. During the balance period, feces, urinary output and spilled food were collected daily and separately for each rat and frozen at −70 °C. At the end of the balance period, total food intake was determined taking into considerations the amount of spilled unconsumed diet. The frozen rat feces and urinary output were freeze-dried, weighed, ground and analyzed for percent nitrogen content by a CHNS/O Analyzer (Perkin Elmer 2400, Connecticut, U.S.A.). The endogenous or metabolic nitrogen loss was determined from the feces of the rats fed the protein-free diet. After completion of the feeding experiment, the rats were deprived of food for 16 h, weighed and then anaesthetized by using diethyl ether. The liver, spleen, kidneys, heart and stomach were rapidly excised and weighed in order to assess the growth performance of the experimental rats. The weight loss of rats fed the protein-free diet was used for computing the NPR.

Biological indices

The data obtained from the animal experiment were used to calculate the parameters such as nitrogen balance (NB, Equation (1)), true protein digestibility (TD, Equation (2)), biological value (BV, Equation (3)), net protein utilization (NPU, Equation (4)), utilizable protein (UP, Equation (5)) and net protein ratio (NPR, Equation (6)) by employing the following formulae recommended by FAO/WHO (1991):

$$\mathrm{NB} = \mathrm{I} - \mathrm{F} - \mathrm{U}; \quad (1)$$

Table 1. Chemical composition of the diets (g/100 g of diet on dried weight basis) used in the experiments

Ingredients	Diet			
	Control	Protein-free	*H. charoides*	*H. japonica*
H. charoides PCs	–	–	12.0 (10.0)[1]	–
H. japonica PCs	–	–	–	11.8 (10.0)[1]
Casein[2]	11.1 (10.0)[1]	–	0.00	0.00
Dextrinzed cornstarch[3]	13.2	13.2	13.2	13.2
Corn starch[4]	49.8	59.8	49.8	49.8
Sucrose[5]	10.0	10.0	10.0	10.0
AIN minerals mixed[6]	3.50	3.50	3.50	3.50
Corn oil[7]	7.00	7.00	7.00	7.00
AIN vitamins mixed[8]	1.00	1.00	1.00	1.00
Fiber (cellulose)[9]	5.00	5.00	5.00	5.00
Choline bitartrate[10]	0.20	0.20	0.20	0.20
L-Cystine[11]	0.30	0.30	0.30	0.30
Kcal/100 g of diet	395	395	395	395
Gross energy density (KJ g^{-1})	16.6	16.6	16.6	16.6

[1]Values in parentheses are the 'net' protein added into the diets.
[2]Casein (Product No. C7078, Sigma Chemical Co., St. Louis, MO).
[3]Dextrainzed cornstarch (Catalog No. 160175, Teklad Test Diets, 2826 Latham Dr., Madison, WISC).
[4]Corn starch (Kingsford, CPC/AJI (Hong Kong) Ltd., Hong Hong).
[5]Sucrose (Taikoo Sugar Ltd., Hong Kong).
[6]AIN Mineral Mix (AIN-93G-MX, Nutritional Biochemicals).
[7]Corn oil (CPC International Inc., U.S.A.).
[8]AIN Vitamin Mix (AIN-93G-VX, Nutritional Biochemicals).
[9]Cellulose (Catalog No. 160390, Teklad Test Diets, 2826 Latham Dr., Madison, WISC).
[10]Choline bitartrate (Product No. C1629, Sigma Chemical Co., St. Louis, MO).
[11]L-Cystine (Product No. C8775, Sigma Chemical Co., St. Louis, MO).

$$\mathrm{TD} = \frac{\mathrm{I} - (\mathrm{F} - \mathrm{Fk}) \times 100}{\mathrm{I}}; \quad (2)$$

$$\mathrm{BV} = \frac{\mathrm{I} - (\mathrm{F} - \mathrm{Fk}) - (\mathrm{U} - \mathrm{Uk})}{\mathrm{I} - (\mathrm{F} - \mathrm{Fk})}; \quad (3)$$

$$\mathrm{NPU} = \frac{\mathrm{BV} \times \mathrm{TD}}{100}; \quad (4)$$

$$\mathrm{UP} = \frac{\mathrm{NPU} \times \text{protein content (g kg}^{-1} \text{ of diet on dry weight basis)}}{100}; \quad (5)$$

$$\mathrm{NPR} = \frac{\text{weight gain of test group + weight loss protein-free group}}{\text{weight of test protein consumed}}. \quad (6)$$

The abbreviations used were I (nitrogen intake), F (fecal nitrogen), Fk (metabolic or endogenous fecal nitrogen), U (urinary nitrogen) and Uk (metabolic or endogenous urinary nitrogen). Body weight gain and protein intake were expressed as grams per rat per day while I, F, Fk, U and Uk were expressed as milligrams per rat per day.

Statistical analysis

All data were presented as mean values ± S.D. The results of all mean values were analyzed by one-way ANOVA and Tukey-HSD at $p < 0.05$ (Wilkinson, 1988) to detect significant differences among groups.

Results and discussion

Protein quality of seaweed PCs

Protein quality in food refers to the ability of a protein to support body growth and maintenance (Wardlaw & Insel, 1993). The biological and chemical evaluation of protein quality is a key factor in the search for new protein sources as well as in the development of food proteins (Carias et al., 1998). A major factor determining protein quality is the amino acid profile. Another factor, which is as important as amino acid profile, is protein digestibility. Even with an excellent amino acid profile, a protein would have a low

Table 2. Food intake, protein intake, weight gain, and NPR of rats fed with seaweed PCs diets[1]

Diet	Food intake (g rat^{-1} day^{-1})	Protein intake (g rat^{-1} day^{-1})	Weight gain (g rat^{-1} day^{-1})	NPR[2]
Control	11.6 ± 1.10^{a}	1.16 ± 0.11^{a}	5.63 ± 0.63^{a}	3.81 ± 0.35^{a}
H. charoides PCs	10.5 ± 0.37^{a}	1.05 ± 0.04^{a}	4.63 ± 0.63^{ab}	3.24 ± 0.48^{a}
H. japonica PCs	10.1 ± 1.02^{a}	1.02 ± 0.10^{a}	4.50 ± 0.41^{b}	3.25 ± 0.10^{a}

[1]Data are mean values of four determinations ± SD. Means in columns with different superscripts (a–b) are significantly different from one another ($p < 0.05$, one-way ANOVA, Tukey-HSD).
[2]NPR = Net Protein Ratio.

Table 3. Nitrogen intake, fecal weight, fecal nitrogen and TD of rats fed with seaweed PCs diets[1]

Diet	N intake (mg rat^{-1} day^{-1})	Fecal weight (g rat^{-1} day^{-1})	Fecal N (mg rat^{-1} day^{-1})	TD[2] (%)
Control	185 ± 17.6^{a}	4.50 ± 0.60^{a}	14.0 ± 1.94^{a}	98.2 ± 0.47^{a}
H. charoides PCs	168 ± 5.75^{a}	4.27 ± 0.46^{a}	25.3 ± 1.66^{b}	90.6 ± 1.16^{b}
H. japonica PCs	162 ± 16.7^{a}	4.07 ± 0.32^{a}	27.8 ± 1.11^{b}	90.6 ± 4.57^{b}

[1]Data are mean values of four determinations ± SD. Means in columns with different superscripts (a–b) are significantly different from one another ($p < 0.05$, one-way ANOVA, Tukey-HSD).
[2]TD = True Protein Digestibility.

nutritional value if its digestibility was low due to its poor bioavailability (Bejosano & Corke, 1998). Our recent work indicated that the amount of essential amino acids of the PCs of *H. charoides* and *H. japonica* accounted for 36.2–38.7% of the total amino acids (Wong & Cheung, 2001). Besides, both *Hypnea* PCs were rich in leucine, valine, threonine, aspartic and glutamic acids but lacked cystine. Except for sulphur-containing amino acids and lysine, the levels of all essential amino acids of these two *Hypnea* PCs were higher than those of FAO/WHO requirement pattern (Wong & Cheung, 2001).

One of the best methods to determine the digestibility of protein is through *in vivo* animal feeding studies (Bejosano & Corke, 1998). A common observation during the evaluation of protein quality *in vivo* is a voluntary reduction in food intake in the animals assigned to the test protein when the quality of this protein is lower than that of control (Carias et al., 1998). In this case, the results may be affected, since the protein consumed is partially utilized as a source of energy and less protein is available to be incorporated in new tissues. As a result, the quality of this protein may be underestimated (Kino & Okumara, 1988; Muramatsu, 1990). Therefore, in order to accurately measure the quality of a protein, the food intake of both the experimental and the control groups should be similar (Carias et al., 1998). In this study, since there were no significant differences of food intake in all different diet groups (Table 2), any effect of seaweed PCs on the biological indices would be reliable and independent of the food intake factor.

As all diets contained the same amount of protein (10%, Table 1), the protein and nitrogen intake of rats fed different diets would be proportional to their corresponding food intake. As a result, no significant differences of protein and N intake were obtained between the seaweed PCs and control diet groups (Tables 2 and 3).

Only the weight gain of *H. japonica* PCs diet group was significantly lower than that of the control group (Table 2). However, when the weight loss of protein-free diet group was considered and the weight gain was expressed as per grams of protein intake (i.e. NPR) (Equation (6)), the NPR of the seaweed PCs diet groups did not significantly differ from that of the control group (Table 2). This implied that the proportion of utilizable seaweed PCs from diet that can turn into new protein tissues was similar to that of the casein control (FAO/WHO, 1991).

According to the National Research Council (U.S.A.), the gross energy density needed for weanling rats to grow is 12.55 KJ g^{-1} of diet (NRCN, 1978). Therefore, the gross energy density of all the diets (16.6 KJ g^{-1} of diet, Table 1) in the present study exceeded the minimal growth value. Besides, it is

Table 4. Urinary nitrogen, NB, BV, NPU and UP in rats fed with seaweed PCs diets[1]

Diet	Urinary N (mg rat^{-1} day^{-1})	NB[2] (mg rat^{-1} day^{-1})	BV[3] (%)	NPU[4] (%)	UP[5] (%)
Control	26.0 ± 1.78^{a}	145 ± 16.6^{a}	93.8 ± 1.99^{a}	92.1 ± 1.88^{a}	92.1 ± 1.88^{a}
H. charoides PCs	29.0 ± 5.01^{a}	113 ± 10.0^{b}	89.7 ± 3.77^{a}	81.3 ± 4.29^{b}	81.3 ± 4.29^{b}
H. japonica PCs	26.2 ± 2.08^{a}	108 ± 18.8^{b}	90.6 ± 3.47^{a}	80.1 ± 4.79^{b}	80.1 ± 4.79^{b}

[1]Data are mean values of four determinations ± SD. Means in columns with different superscripts (a–b) are significantly different from one another ($p < 0.05$, one-way ANOVA, Tukey-HSD).
[2]NB = Nitrogen Balance.
[3]BV = Biological Value.
[4]NPU = Net Protein Utilization.
[5]UP = Utilizable Protein.

valuable to note that, the energy density of all diets still exceed the minimal growth value of rat, even though the energy supply is only based on carbohydrate and fat (14.9 KJ g^{-1} diet). This indicated that apart from the protein, all experimental diets could provide sufficient adequate energy from other energy sources for weanling rats to grow. Therefore, dietary proteins are unlikely to break down and release energy to meet the body's need. As a result, the evaluation of protein quality on the test protein would be more accurate, reliable and meaningful (Whitney et al., 1991; Wardlaw & Insel, 1993).

Although the fecal weight and urinary N of seaweed PCs diet groups were not significantly different from those of the control group (Tables 3 and 4), the fecal nitrogen loss of the seaweed PCs diet groups was significantly higher (about 2 folds) than that of the control group (Table 3). Because of this significantly higher fecal nitrogen excretion, significantly lower NB and TD values of seaweed PCs diet groups were obtained as compared with those of the control (Equations (1) and (2)) (Tables 3 and 4). All NB was positive (positive N balance) indicating that there is extra nitrogen for the weanling rats to grow (synthesis new protein tissues) although the amount of this extra nitrogen in control group was significantly higher. Besides, N was mainly lost in feces rather than urine. Furthermore, the significantly lower TD values of seaweed PCs implied that their percentage of intake N that can be absorbed were lower than that of the control (FAO/WHO, 1991).

Comparing with the seaweed PC diet groups, the control group not only possessed significantly higher apparent nitrogen retention (i.e. NB) (Table 4), but also exhibited the significantly highest level of absorbed nitrogen (i.e. TD) (Table 3). As a result, no significant differences between the BV of the seaweed PCs and control diet groups were obtained (Equation (3)) (Table 4). Besides, this suggests that the proportion of absorbed nitrogen that is retained for maintenance or growth in both seaweed PCs and control diet groups were similar (FAO/WHO, 1991).

Table 4 also shows that the NPU and UP of the seaweed PCs diets were significantly lower than those of the control, implying that the proportion of nitrogen intake retained for growth or maintenance as well as the maximum amount of protein that can be utilized in seaweed PCs diet groups were lower than those of the control (FAO/WHO, 1991). Although there were no significant differences in nitrogen intake by all diets groups (Table 3), the result of NPU and UP may probably be due to the significantly higher level of NB in the control group (Equations (4) and (5)) (Table 4).

In conclusion, the overall protein quality of the two seaweed PCs was lower than that of the casein control. This was indicated by their significantly lower levels of true protein digestibility (TD), nitrogen balance (NB), net protein utilization (NPU) and utilizable protein (UP). The intake nitrogen was mainly lost in the feces and not in the urine. This suggested that the lower protein quality of the seaweed PCs would be mainly due to their relatively lower digestibility rather than limited supply of essential amino acids (which resulted in additional N lost in urine) (Whitney et al., 1991; Wardlaw & Insel, 1993). However, the nitrogen retention (for growth and maintenance) of absorbed N from seaweed PCs was similar to that of absorbed casein N, since there were no significant differences in BV of all diets even though the absorbed nitrogen of seaweed PCs was significantly lower than that of the casein. Besides, the protein quality of the two seaweed PCs was similar, as no significant differences

Table 5. Relative weight (g/100 g of body weight) of liver, kidney, spleen, stomach and heart of rats fed with seaweed PCs diets[1]

Diet	Liver	Kidney	Spleen	Stomach	Heart
Control	4.15 ± 0.32^{a}	0.81 ± 0.02^{a}	0.22 ± 0.03^{ab}	0.59 ± 0.01^{a}	0.48 ± 0.03^{a}
H. charoides PCs	3.68 ± 0.11^{a}	0.78 ± 0.04^{a}	0.18 ± 0.01^{a}	0.59 ± 0.05^{a}	0.48 ± 0.06^{a}
H. japonica PCs	3.81 ± 0.39^{a}	0.78 ± 0.06^{a}	0.18 ± 0.01^{a}	0.61 ± 0.02^{a}	0.51 ± 0.03^{a}

[1]Data are mean values of four determinations ± SD. Means in columns with same superscripts do not differ significantly from each other ($p > 0.05$, one-way ANOVA, Tukey-HSD).

were found in all the biological indices between the two seaweed PCs.

The weight of major organs

Variations in the weight and appearance of livers in rats fed with different diets were related to the difference in the amount of cholesterol and oil added into the diets (Beynen et al., 1986; Dadai et al., 1996). The absence of significant differences in the weight of liver of all different diets groups (Table 5) may be explained by the fact that the amount of corn oil added in each test diet was identical (5%, Table 1). Besides, the weights of kidney, spleen, stomach and heart of seaweed PCs diet groups also were not significantly different from those of the control group (Table 5). This suggested that consumption of these two seaweed PCs would not cause any adverse effect on the weight of these major organs.

The protein quality of the seaweed PCs was comparable to that of common plant PCs obtained from legumes and cereals. Rozan et al. (1997) reported that like seaweed PCs diet groups, the N intake of soybean PCs diet group was similar to that of casein control group and the fecal N of the soybean and sweet lupine seed PCs diet groups were significantly lower than that of the casein control group. Besides, the TD of the seaweed PCs diets (over 90%, Table 3) was comparable to that of pea PCs (92.6%), faba bean PCs (93.0%), soybean PCs (81.2%) (Fernández-Quintela et al., 1998) and rice bran PCs (84.1%) (Prakash, 1996). Furthermore, similar to the seaweed PCs diet groups, the NB and NPU of rats fed with pea, faba and soybean PCs were significantly lower than that of the casein control (Fernández-Quintela et al., 1998). On the contrary, however, a reduction in the weight of liver and spleen has been reported in rats fed with pea and soybean PCs (Fernández-Quintela et al., 1998).

Although the protein quality of the two seaweed PCs was lower than that of the casein control based on the biological indices mentioned, it was comparable to that of other common plant PCs. Besides, both seaweed PCs had no adverse effect on the weight of some major organs and together with their good protein quality; they had the potential to be developed into a new protein source. Further investigation on the functional properties of these two seaweed PCs is currently underway and should provide a better appreciation of the potential of seaweed as a new protein source in animal and human diets.

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