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Nutritional value of proteins from edible seaweed *Palmaria palmata* (Dulse)

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Palmaria palmata *(Dulse) is a red seaweed that may be a potential protein source in the human diet. Its protein content, amino acid composition, and protein digestibility were studied with algae collected every month over a 1-year period. Significant variations in protein content were observed according to the season: The highest protein content (21.9 ± 3.5%) was found in the winter–spring period and the lowest (11.9 ± 2.0%) in the summer–early autumn period. Most of the essential amino acids were present throughout the year. After 6-hour in vitro digestion in a cell dialysis using porcine pepsin and porcine pancreatin, the digestibility of proteins from* Palmaria palmata *crude powder, represented by dialyzed nitrogen, was estimated at 29.52 ± 1.47%. Relative digestibility was 56%, using casein hydrolysis as 100% reference digestibility. In vitro digestibility of proteins extracted in water was analyzed by sodium dodecylsulfate polyacrylamide gel electrophoresis using either bovine trypsin, bovine chymotrypsin, pronase from* Streptomyces griseus, *or human intestinal juice. Dulse proteins were hydrolyzed to a limited extent, which confirmed a rather low digestibility. Hydrolysis rate was higher with trypsin and lower with chymotrypsin compared with the two other enzymatic systems, pronase and intestinal juice, respectively. The association of algal powder and protein extract to casein and bovine serum albumin, respectively, produced a significant decrease in the hydrolysis rate of the standard proteins. In conclusion, the digestibility of* Palmaria palmata *proteins seems to be limited by the algae non-proteic fraction.* (J. Nutr. Biochem. 10:353–359, 1999) © *Elsevier Science Inc. 1999. All rights reserved.*

Keywords: seaweed; *Palmaria palmata*; nutritional value; protein; amino acid; *in vitro* digestibility

Introduction

It is well known that red seaweed possesses a high protein level. *Porphyra tenera* and *Palmaria palmata* contain especially high levels, with as much as 47.5%[1] and 30%,[2] respectively, found in their fronds. However, there are few data about the variation of the amino acid composition of these proteins according to the season. In addition, little information is available about the nutritional value of red algal proteins. Previous studies have evaluated the nutritional value of these proteins by comparing their amino acid composition to that of egg proteins.[3] Few studies concerning *in vitro* digestibility of red algal proteins are available. The relative digestibility of *P. tenera* proteins has been found to be approximately 50% with pancreatin and at least 70% with pronase; by comparison, when casein is used as a reference, digestibility is 100%.[1,4] However, these results were obtained using alkali-soluble proteins rather than native proteins as substrate.[1]

In the present study, the nutritional value of *P. palmata* was determined by analysis of seasonal variations in the protein and amino acid contents and evaluation of protein digestibility. We evaluated the *in vitro* digestibility of two

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types of protein extracts from *P. palmata*—crude powder and water-soluble protein extract—and compared them with two reference proteins—bovine serum albumin (BSA) and casein—taking into account the influence of algal powder and protein extract on proteolysis. Two experimental conditions were used: hydrolysis in a dialysis cell system[5] and sodium dodecylsulfate polyacrylamide gel electrophoresis (SDS-PAGE) analysis of hydrolyzed proteins.

Methods and materials

Materials

The *P. palmata* specimens were collected monthly (except in August) during 1996 at Belle Ile (located on the French Brittany coast). Epiphytes were removed and samples were successively rinsed with seawater and distilled water. The algae were stored at −20°C.

Intestinal juice was collected during duodenal endoscopy.

Casein (bovine milk), BSA, pepsin (porcine stomach mucosa, 1:60,000, activity 3,200 units/mg of proteins), pancreatin (porcine pancreas, activity at least equivalent to USP specifications), trypsin (bovine pancreas, type III, activity 10,000–13,000 BAEE units/mg of proteins), chymotrypsin (bovine pancreas, type II, activity 40–60 units/mg of proteins), and pronase (*Streptomyces griseus*, protease type XIV, activity 4 units/mg of proteins) were purchased from Sigma Chemical Co. (St. Louis, MO USA).

Obtaining algal powder

The algae were freeze-dried and immediately ground with a Waring blender and then with an ultracentrifuge type grinder (Retsch) with a 1-mm screen. The algal powder was the raw material used for this study.

Protein determination and monitoring of protein level

The protein determination of 11 *P. palmata* specimens collected monthly (except in August) was carried out in triplicate using the Kjeldhal method (N × 6.25).[3] Mean values and standard deviation of measurements ($n = 3$) were determined for each specimen. The protein concentration in the solution was determined with bicinchoninic acid (BCA) protein reagent assay (Pierce, Rockford, IL USA) according to the manufacturer's instructions.[6] BSA was used as the protein standard.

Amino acid composition

The proteins from 10 *P. palmata* specimens collected monthly (except in August) were hydrolyzed with 6 N HCl at 110°C for 24 hours. The amino acid composition was determined by high performance liquid chromatography (HPLC) using a cation exchange column (Pickering type 316 SS, Pickering Laboratories, Mountain View, CA USA) and a ninhydrin derivation post-column. The amino acid standard H (Pierce) was used to determine the amino acid concentrations of the samples. Yearly average and standard deviations were determined for each amino acid.

In vitro *digestibility of proteins from algal powder*

Algal powder and casein (40 mg total nitrogen) were subjected to two-step *in vitro* digestion into a "digestion cell."[5] This dialysis cell method appears to be very valuable for mimicking luminal protein degradation in the small intestine.[7] Digestion was carried out on the *P. palmata* specimen collected in February 1996, which contained a high level of proteins. Briefly, the 30-minute pepsin predigestion (E/S: 1/250) was followed by a 6-hour pancreatin proteolysis (E/S: 1/25) in a dialysis cell (Model Ser II, Quebec, Canada) using a 1,000-MW cut-off dialysis bag. Digestibility of protein samples was calculated by means of the following formula and was represented by nitrogen digestibility (ND):

$$\text{ND (\%)} = \text{N in dialysate (mg)/N in protein sample (40 mg)} \times 100$$

Relative nitrogen digestibility of protein samples was calculated using casein hydrolysis as a 100% reference digestibility.

Digestion of casein with 1/6 or 1/4 (w/w) algal powder (40 mg total nitrogen) also was carried out to study the influence of algal powder on casein digestibility.

All these *in vitro* experiments were performed in triplicate and nitrogen determination was carried out in duplicate by Kjeldahl method. Statistical analysis of the results was determined by analysis of variance (ANOVA) using Statview II system on MacIntosh computer. Mean values and standard deviation of measurements ($n = 6$) were calculated for each experiment and statistical significance was determined by the calculation of *P*-values.

Obtaining algal protein extract

The whole procedure was carried out at 4°C or below. Algal powder (10 g) was suspended in ultrapure water (1,000 mL) to make cell lysis by osmotic shock and facilitate protein extraction.[8] After an ultrasonic bath for 1 hour, the suspension was stirred overnight. The homogenate was clarified by centrifugation at 10,000 g for 1 hour. The pellet fraction was suspended in 200 mL of ultrapure water and subjected to a second extraction procedure. Both supernatants were pooled, brought to 80% ammonium sulfate saturation, and agitated for 1 hour. The mixture was centrifuged at 20,000 g for 1 hour. The protein precipitate after centrifugation was suspended in a minimal volume of ultrapure water and dialyzed overnight against ultrapure water. The dialyzed extract was freeze-dried.

In vitro *digestibility of proteins from algal extract*

Digestion was performed in the presence of either bovine trypsin, bovine chymotrypsin, pronase, or human intestinal juice with the protein extract from the *P. palmata* specimen that had been collected in November 1996 and had contained a high protein level. All reactions were carried out at 37°C, pH 7.4. The proteolytic activity of the different enzymatic systems was studied at various incubation times and evaluated by protein patterns on SDS-PAGE.

The protocol was adapted from the procedure described by Singh *et al*.[9] Briefly, the algal protein freeze-dried extract was dissolved in ultrapure water. After incubation at 37°C for 15 minutes with gentle rocking, the protein extract was incubated with either 1/10 (w/w) trypsin, 1/10 (w/w) chymotrypsin, 1/10 (w/w) pronase, or 1/100 (v/v) human intestinal juice. Aliquots of digests were withdrawn at 1, 10, 30, 60, and 120 minutes, and added to a Tris-HCl buffer 30 mM, pH 8.0, EDTA 3 mM, SDS 3% (w/v), DTT 120 mM, glycerol 30% (v/v), and bromophenol blue 0.05% (w/v), which was immediately followed by heating at 100°C for 3 minutes. Aliquots of digests were then analyzed by polyacrylamide gel electrophoresis.

A control hydrolysis procedure also was performed with BSA under the above-mentioned conditions.

Polyacrylamide gel electrophoresis. SDS-PAGE was performed using a Mini-protean II electrophoresis unit (Bio-Rad, Hercules, CA USA) with stacking gels of 4% and separating gels of 12% acrylamide in Tris-HCl 25 mM, pH 8.3, glycine 0.18 M, and SDS 0.1%. The separation was carried out at 30 mA for 90 minutes. The

following polypeptides were used as molecular mass markers: phosphorylase b (94 kDa), albumin (67 kDa), ovalbumin (43 kDa), carbonic anhydrase (30 kDa), trypsin inhibitor (20.1 kDa), and lactalbumin (14.4 kDa). (All measurement were determined using LMW calibration kit proteins, Pharmacia, Uppsala, Sweden.) After separation, protein bands of algal protein extract and *BSA* digestion were detected by silver staining and Coomassie blue, respectively.

Silver staining. Proteins were fixed with 40% methanol and 10% acetic acid for 1 hour. The gel then was washed with 30% ethanol for three times for 20 minutes. Proteins were reduced with 0.02% sodium thiosulfate for 1 minute, washed with ultrapure water three times for 20 sec, and stained with 0.2% silver nitrate and 0.02% formaldehyde for 20 minutes. The gel was washed, developed with 3% carbonate, 0.05% formaldehyde, and 0.0005% sodium thiosulfate for 3 to 5 minutes and washed again. Coloration was stopped with 0.5% glycine for 5 minutes and the gel was washed with ultrapure water twice for 30 minutes. The gel was then dried for 1 hour (Gel dryer, Biorad).

Coomassie blue. Intensity of the 66-kDa band corresponding to BSA was estimated using Im1d software.

Detection of glycoproteins from algal protein extract

Algal protein extract (1 μg, 10 μg of proteins) was applied to nitrocellulose (0.2 μm trans-blot transfer medium, Bio-Rad) using the Bio-Slot microfiltration apparatus (Bio-Rad). Glycoproteins were detected using the DIG-glycan differentiation kit (Boehringer Mannheim) according to the manufacturer's instructions.

Five lectins that selectively recognize the terminal sugars were used. These were Galanthus Nivalis Agglutinin (GNA), Sambucus Nigra Agglutinin (SNA), Maackia Amurensis Agglutinin (MAA), Peanut Agglutinin (PNA), and Datura Stramonium Agglutinin (DSA). GNA, SNA, MAA, PNA, and DSA recognize terminal mannose, sialic acid linked α(2-6) to galactose, sialic acid linked α(2–3) to galactose, galactose-β(1-3)-N-acetyl-galactosamine, and galactose-β(1-4)-N-acetyl-glucosamine, respectively.

Blots were incubated for at least 30 minutes in blocking solution, washed twice for 10 minutes each in 0.05 M Tris-HCl and 0.15 M NaCl, pH 7.5 (TBS), and washed once in TBS, 1 mM $MgCl_2$, 1 mM $MnCl_2$, and 1 mM $CaCl_2$. Slots then were incubated for 1 hour with different digoxigenin-labeled lectins, washed three times for 10 minutes each in TBS, incubated for 1 hour with anti-digoxigenin-alkaline phosphatase, and washed three times for 10 minutes each in TBS. Slots then were stained for a few minutes with 4-nitro blue tetrazolium chloride/5-bromo-4-chloro-3-indolyl-phosphate (NBT/X-phosphate) and rinsed several times with distilled water to stop the reaction. Carboxypeptidase Y, transferrin, fetuin, and asialofetuin were used as reference glycoproteins.

Results

Protein content

Eleven *P. palmata* specimens collected monthly during 1996 at Belle Ile were analyzed for protein content. Based on the nitrogen level, the protein amount of these specimens varied from 9.7 to 25.5% of the dry mass (*Figure 1*), with a yearly average of 18.3 ± 5.9%. A seasonal variation of protein content was clearly observed, with maximum values (21.9 ± 3.5%) occurring during the winter–spring period and lower levels (11.9 ± 2.0%) during the summer–early autumn period.

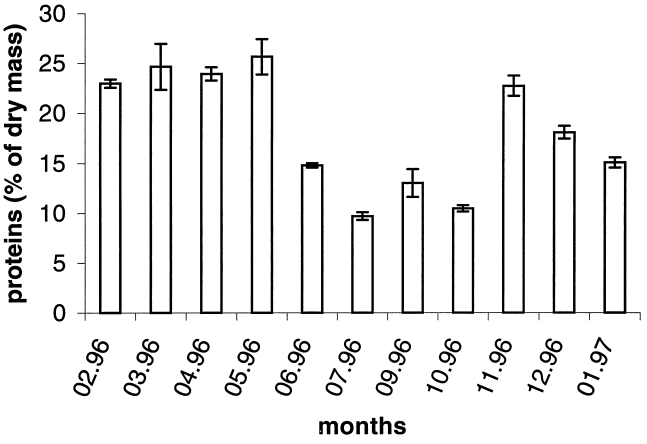

Figure 1 Seasonal variation of protein content from *Palmaria palmata*. Mean values and standard deviation of measurements ($n = 3$) are indicated for each specimen.

Amino acid composition

The amino acid composition of proteins was determined in 10 *P. palmata* specimens collected in different months during 1996 (*Table 1*). All 10 samples were characterized by a high amount of aspartic acid and glycine and, to a lesser extent, glutamic acid, leucine, and valine. Dulse proteins also contained alanine, serine, arginine, and phenylalanine in lower quantities, and were poor in methionine, hydroxyproline, proline, and histidine. Cystine was absent. Acidic amino acids (28.5 ± 8.7% of total amino acids) were more dominant than basic amino acids (7.8 ± 6.2%). The essential amino acids of dulse proteins accounted for 26.1 to 50.0% of total amino acids, with an average of 35.8%.

Some seasonal fluctuations were observed. Glutamic acid, serine, and alanine were present in a relatively high amount during late winter, spring, September, and October, and were absent the rest of the year. Arginine disappeared in spring, lysine in spring and July, histidine in spring and summer, and hydroxyproline in February, spring, September, and October. Proline appeared only in February, September, and October.

In vitro *hydrolysis of proteins from algal powder*

The algal powder of the *P. palmata* specimens collected in February 1996 at Belle Ile was subjected to *in vitro* digestion in the dialysis cell. Protein digestibility was expressed as a percentage of dialyzed nitrogen. Nitrogen digestibility of *P. palmata* (29.52 ± 1.47%) during a 6-hour period was significantly lower ($P < 0.001$) than that of casein (53.24 ± 4.03%; *Figure 2*). Using casein hydrolysis as a 100% digestibility reference, the relative nitrogen digestibility of *P. palmata* was 56%. Furthermore, the nitrogen digestibility of casein after 6 hours of digestion was reduced significantly from 53.24 to 40.35 ± 4.18% ($P < 0.001$), when adding 1/4 (w/w) algal powder (*Figure 2*).

In vitro *hydrolysis of proteins from algal extract*

The protein extract of the *P. palmata* specimens collected in November 1996 at Belle Ile was subjected to *in vitro*

Table 1 Seasonal variation of amino acid composition from proteins of *Palmaria palmata* (g/100 g amino acids)

	Palmaria palmata										Average*			
	02/96	04/96	05/96	06/96	07/96	09/96	10/96	11/96	12/96	01/97	A	SD	Egg[10]	Soya[11]
Essential amino acids														
Ile	4.0	3.6	1.9	4.4	6.4	0.0	3.1	4.6	4.1	4.9	3.7	±1.7	5.4	5.1
Leu	7.1	9.4	8.2	0.0	13.6	0.0	6.7	9.0	8.3	9.0	7.1	±4.0	8.6	7.6
Lys	5.3	0.0	0.0	0.0	0.0	6.1	4.3	5.9	5.5	5.7	3.3	±2.7	7.0	6.1
Met	2.0	3.7	2.5	3.4	3.8	1.8	1.6	2.5	2.7	2.9	2.7	±0.7		
Cys	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	±0.0		
Met+Cys	2.0	3.7	2.5	3.4	3.8	1.8	1.6	2.5	2.7	2.9	2.7	±0.7	5.7	2.7
Phe	4.1	0.0	6.6	3.7	10.0	0.0	5.0	6.8	6.8	7.8	5.1	±3.2		
Tyr	3.9	0.0	0.0	4.5	6.4	0.0	0.0	5.4	6.4	7.0	3.4	±2.9		
Phe+Tyr	8.0	0.0	6.6	8.2	16.4	0.0	5.0	12.2	13.2	14.8	8.4	±5.6	9.3	8.4
Thr	5.4	6.9	8.7	0.0	0.0	6.7	8.0	0.0	0.0	0.0	3.6	±3.7	4.7	4.1
Val	7.1	7.9	0.0	10.2	9.8	5.7	5.2	8.1	7.0	7.7	6.9	±2.7	6.6	5.2
Total	38.9	31.5	27.9	26.2	50.0	20.3	33.9	44.3	40.8	45.0	35.8	±9.0	47.3	39.2
Nonessential amino acids														
His	1.2	0.0	0.0	0.0	0.0	0.0	0.0	1.4	1.1	1.1	0.5	±0.6		
Asp	12.7	15.7	18.6	19.8	10.3	15.8	12.2	26.8	27.9	25.6	18.5	±6.0		
Glu	12.1	20.7	17.6	19.8	0.0	15.6	13.5	0.0	0.0	0.0	9.9	±8.5		
hPro	0.0	0.0	0.0	0.0	8.4	0.0	0.0	4.5	4.9	5.2	2.3	±3.0		
Pro	6.6	0.0	0.0	0.0	0.0	6.9	4.7	0.0	0.0	0.0	1.8	±2.7		
Ser	7.4	10.7	18.3	12.7	0.0	7.7	5.7	0.0	0.0	0.0	6.3	±6.0		
Gly	6.5	9.3	8.7	8.4	22.5	15.3	9.0	19.1	18.6	15.6	13.3	±5.3		
Ala	8.7	12.2	8.9	13.3	0.0	11.1	12.6	0.0	0.0	0.0	6.7	±5.6		
Arg	6.1	0.0	0.0	0.0	8.9	7.4	8.4	6.1	6.9	7.4	5.1	±3.5		

Note: Tryptophane was not determined.
*Yearly average (A) and standard deviation (SD) are indicated for each amino acid.

digestion using either intestinal juice, pronase, trypsin, or chymotrypsin prior to SDS-PAGE analysis (*Figure 3*). Trypsin digestion was found to be higher than the others and chymotrypsin digestion found to be lower compared with those of the enzymatic systems pronase and intestinal juice. The electrophoresis pattern of the digests indicate that the major 55-kDa protein band was readily hydrolyzed by intestinal juice, pronase, and trypsin. The degradation was already significant after only 10 minutes of digestion, and some peptides of intermediate size were formed. The 55-kDa protein band was almost completely hydrolyzed after 120 minutes of digestion. However, this protein band was somewhat resistant to chymotryptic hydrolysis. The rate of digestion with chymotrypsin was lower than with the other enzymatic systems tested and a fraction of the protein band was left intact after 120 minutes of incubation. Comparison of the protein pattern of digests and unhydrolyzed extracts shows that the different enzymatic systems had a limited effect on the 10- to 30-kDa protein bands after 120 minutes of incubation. Finally, dulse proteins in general were hydrolyzed to a limited extent, no matter which enzyme was tested. The glycosylation of proteins was studied by lectin blotting, because glycosylation may have a protective effect. This blotting was negative with five lectins.

The efficiency of the hydrolysis procedure was assessed using BSA as a control protein. BSA was hydrolyzed in small peptides, no matter which enzymatic solution was tested. Compared with BSA alone, the association of BSA to a protein extract from *P. palmata* produced a significant decrease in the hydrolysis rate with trypsin, chymotrypsin, and intestinal juice, whereas a similar rate was maintained with pronase (*Figure 4*).

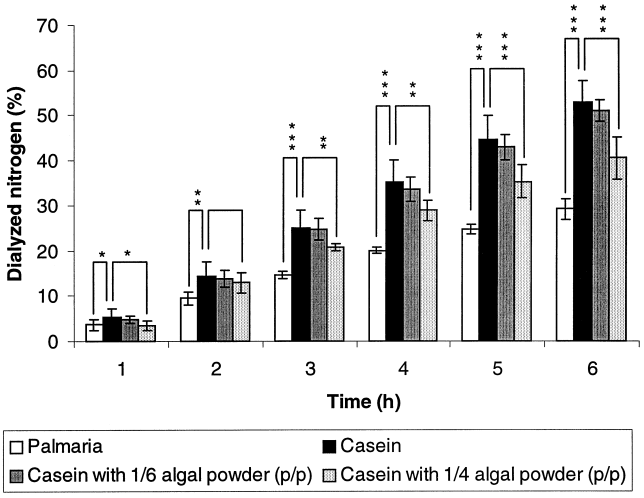

Figure 2 Release of nitrogen from casein, *Palmaria palmata* powder, casein in presence of one sixth algal powder, and casein in presence of one quarter algal powder by pancreatin in the digestion cell according to Savoie and Gauthier.[5] Samples were previously hydrolyzed for 30 minutes with pepsin. Mean values and standard deviation of measurements ($n = 6$) are indicated for each data point. Statistical significance of the results is indicated by P-value (*$P < 0.03$, **$P < 0.01$, and ***$P < 0.0001$).

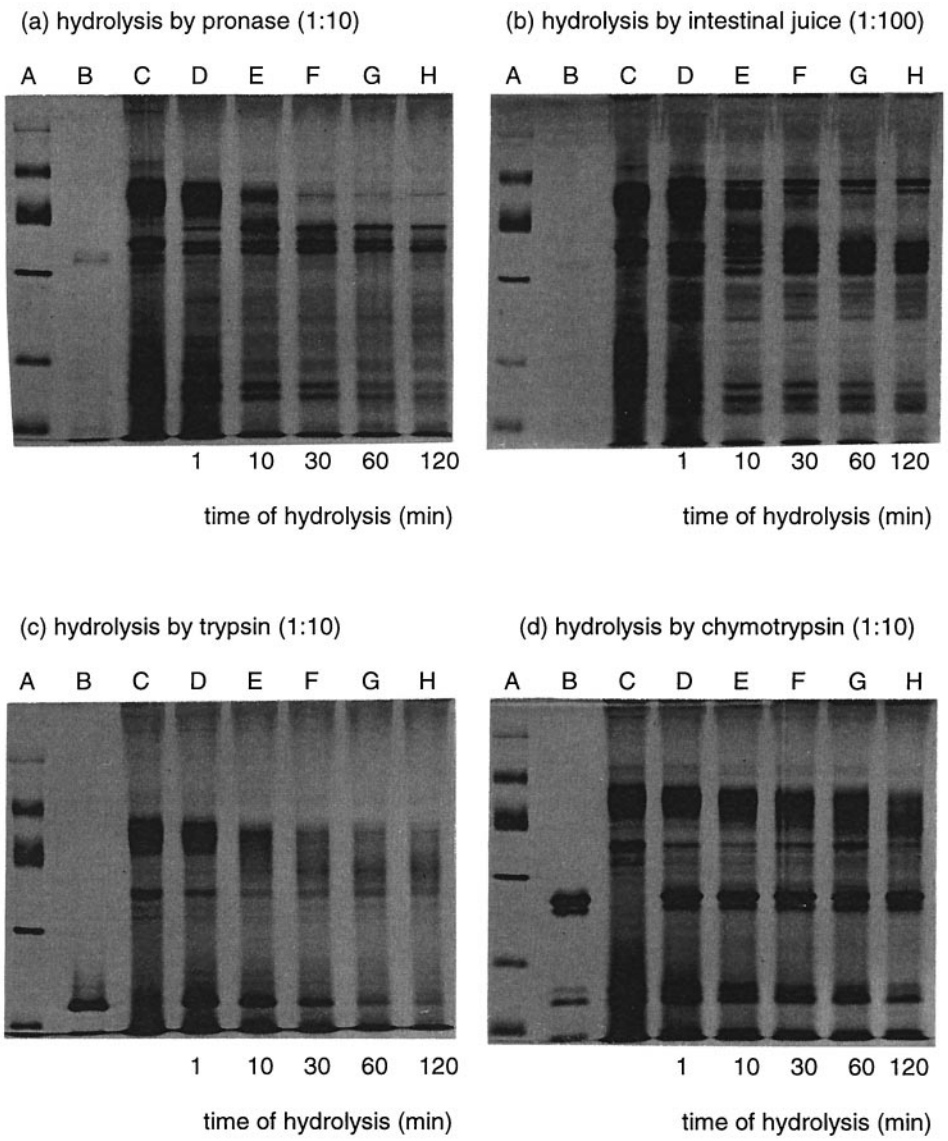

Figure 3 Sodium dodecylsulfate polyacrylamide gel electrophoresis analysis of hydrolysates of the *Palmaria palmata* proteins by the different enzymatic systems. Electrophoresis was run in a 12% acrylamide gel (30 mA per gel) and protein bands were detected by silver staining. *Lane A*: standard proteins (phosphorylase b 94 kDa, albumin 67, ovalbumin 43, carbonic anhydrase 30, soybean trypsin inhibitor 20.1, lactalbumin 14.4); *Lane B*: reference enzymatic solution; *Lane C*: reference extract of *Palmaria palmata* proteins; *Lane D-H*: hydrolysates of *Palmaria palmata* proteins at 0, 10, 30, 60, and 120 minutes.

Discussion

The deficient proteinous intake of many developing populations had led to the search for cheap proteins from different vegetable sources. Vegetable proteins represent 80% of provided nitrogen in developing countries and less than 50% in industrialized countries.[12] The nutritional value of vegetable proteins depends on essential amino acid content and digestibility.

Amino acid composition

Amino acid content should be compared with human amino acid requirements when assessing the nutritional value of *P. palmata*. According to Dupin *et al.*[13] and Friedman:[14] three factors are important: amino acid balance, essential relative amino acid content compared with the egg protein reference, and ratio of essential amino acids. *P. palmata* proteins are of high quality in this respect because, as shown in this study, the essential amino acids represented as much 26 to 50% of total amino acids; the essential amino acid profile was close to that of egg protein. The amino acid content compared favorably with leguminous plants such as soya (*Table 1*), except for the relative lack of lysine in summer and threonine in summer and early winter. In fact, threonine, lysine, tryptophane, and sulfur amino acids are usually limiting amino acids for algal proteins but the amino acid general store of the algal proteins remain higher than those described in land plants.[1]

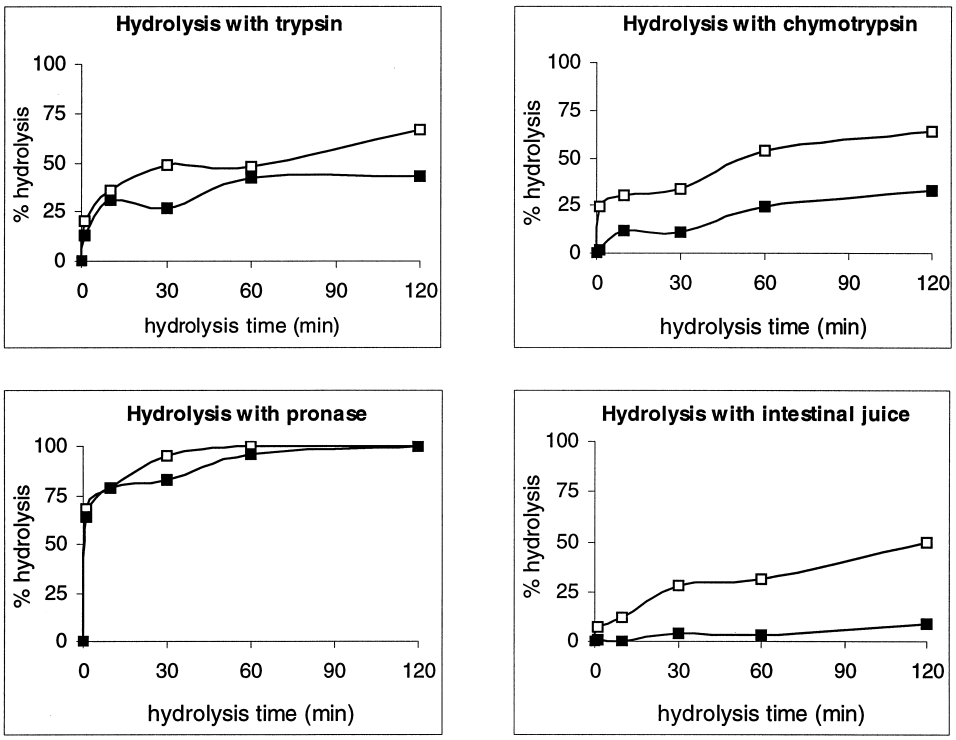

Figure 4 Influence of algal protein extract on bovine serum albumin (BSA) hydrolysis. Hydrolysis of BSA alone (□) and BSA with 1/10 (w/w) algal protein extract (■).

Seasonal fluctuations of the amino acid composition recorded in this study suggest seasonal changes in the types of proteins present. These changes could be explained by variations of environmental conditions such as sunlight intensity and nitrogen deprivation.[15]

The main findings of the amino acid composition of *P. palmata* proteins described here are in agreement with previous studies. Aspartic and glutamic acids often are found to be the most abundant amino acids.[2,16] Dulse proteins also are reported to be rich in glycine, alanine, leucine, valine, and arginine, and poor in histidine, tryptophane, cystine, and methionine.[2,17] The predominance of acidic amino acids over basic amino acids is typical of red seaweed.[1,18]

Protein content

In comparison with most seaweeds traditionally consumed in Japan, except *P. tenera*,[1] *P. palmata* contains relatively high levels of proteins (*Table 2*). In the winter–spring period, increased protein contents of *P. palmata* (21.9 ± 3.5%) are comparable with those of high protein vegetables, in particular leguminous plants such as Soya (25% of proteins).

Seasonal variations of protein content in red seaweed were previously described: The highest protein content is found during winter and early spring and the lowest during summer and autumn.[19–21] A correlation was noted between the nitrogenous nutrients in seawater and the protein content of red seaweed.[15,20,21] Basically, these studies have shown that maximum values of proteins (winter and early spring) were found when maximum nutrients were available and vice versa. In tank culture, *P. palmata* grown in nitrogen-depleted water showed a rapid decline of protein content.[2] On the other hand, intense summer sunlight also could cause destruction of proteins.[15]

In vitro *digestibility of algal proteins*

Even though the amino acid profile is important in evaluating the nutritional value of proteins, the digestibility of those proteins is the primary factor of the availability of their amino acids. Some *in vitro* measurements of algal protein digestibility have been reported.[1] For instance, *in vitro* digestibility of proteins from *P. tenera*, calculated using casein hydrolysis as a 100% reference digestibility, was 73% with pronase and 50% with pancreatin. However, these studies were carried out on alkali-soluble proteins, which represented 7 to 20% of crude proteins, and not on native proteins.

Table 2 Protein content of some algae compared with *Palmaria palmata**

Algae	Protein (% of frond)
Porphyra tenera	47.5
Analipus japonicus	23.7
Grateloupia turuturu	20.0
Palmaria palmata	18.3 ± 5.9
Ulva pertusa	17.5
Laminaria japonica	15.6
Codium fragile	15.6
Eisenia bicyclis	13.1
Undaria pinnatifida	12.5

*(Fujiwara-Arasaki *et al*. 1984, and present study)

Our study is the first to evaluate the digestibility of *P. palmata* proteins. We showed that *in vitro* digestibility of *P. palmata* proteins was rather moderate when both algae powder and water-soluble protein extract algae preparations were tested. This limited rate of hydrolysis may be due to an inhibiting effect of either trypsin inhibitor, phenolic compounds, and/or fiber, in particular polysaccharides; a protecting effect of protein by glycosylation also may be involved in this limited proteolysis. In fact, some studies have showed that for high rates of glycosylation, the rates of hydrolysis decreased with trypsin and chymotrypsin[22] and that elimination of carbohydrates bound to proteins increased the action of trypsin.[23] The most likely hypothesis is that the presence of an inhibitor decreased the proteolytic activity, because no glycoprotein was detected by lectin blotting in the protein extract of *P. palmata*, excluding the hypothesis of a protection of proteins by a carbohydrate core. This hypothesis is supported by decreases in casein hydrolysis in the presence of algal powder. The decrease in digestibility may be due not only to a dilution of the highly digestible casein with a less digestible algal powder, but also to the presence of some inhibitors. Algal protein extract also appears to have an inhibiting effect on BSA hydrolysis when trypsin, chymotrypsin, and intestinal juice are used. In contrast, algal protein extract had only a limited effect when BSA was subjected to pronase, which is a nonspecific protease from *S. griseus*. The inhibiting effect of phenolic compounds is probably not found in *P. palmata*, which possesses low levels of phenols.[24] On the contrary, *P. palmata* contains a high level of fibers (at least 30% of the dry mass),[25] in particular polysaccharides, notably cell wall structural polysaccharides.[2,24] The major polysaccharide of *P. palmata* is a water-soluble xylan. The presence of polysaccharides and their interaction with proteins may reduce the accessibility of proteins to proteolysis, which causes decreased digestibility. The influence of fibers on algal protein digestibility should be investigated. The use of this algae as a protein source for the human diet requires the definition of an adequate extraction procedure. The goal of this procedure would be to separate the protein extract from the fibers.

Conclusion

P. palmata is a potentially good source of proteins (10–26% of the dry mass), because it contains most of the essential and nonessential amino acids. *In vitro* digestibility of these proteins is fairly moderate, perhaps due to the presence of polysaccharides.

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