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In vitro Digestibility of Algal Proteins

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Experiments were carried out to complement previously gained basic knowledge of algal proteins as food sources. After five hours' digestion of the alkali soluble proteins of algae *in vitro*, the digestibility with pepsin was found lower (15-56%) than these with two other enzymes, pancreatin (37-68%) and pronase (59-94%), when studies using eight edible marine algae, *Codium fragile, Ulva pertusa, Eisenia bicyclis, Undaria pinnatifida, Laminaria japonica, Analipus japonicus, Grateloupia turuturu,* and *Porphyra tenera.* Under the same condition, and when incubated for twenty-four hours, the protein digestibility became 42.4 to 90.9% with pepsin, 60.2 to 100% with pancreatin and 81.0 to 100% with pronase, respectively. The digestibility of alkali soluble proteins was particularly better than that of dried pulverized algae.

Key Index Words: *Algal proteins, alkali soluble protein,* in vitro *digestibility, marine algae.*

The value of marine algae as food has recently been re-evaluated in the hope that it may solve future food shortages. In Japan, more than one hundred species of marine algae have been used traditionally as food. Even today, the Japanese consume large quantities of marine algae such as *Laminaria, Undaria, Eisenia, Hijikia, Analipus, Monostroma, Enteromorpha, Ulva, Porphyra, Meristotheca* and *Gelidium.* Among these, the four species, *Porphyra tenera, Laminaria japonica, Undaria pinnatifida* and *Monostroma* sp. are artificially cultivated at present on a commercial scale in Japan.

It is well known that marine algae are a good source of carbohydrates, vitamins and minerals, and are a relatively high source of protein in human nutrition. Carbohydrates of marine algae are not digested by human intestinal enzymes. For this reason, they do not provide the human system with a source of calories. Moreover, they are low in fats. The edible marine algae, however, are not only predominant sources of such vitamins as A (β-carotene), B_1, B_2, B_6, B_{12}, C and niacin, but also more important sources of calcium and iron than vegetables and fruits found in the traditional human food. These facts indicate that algae have a good potential to promote health by way of nutritional and weight-reducing effects, and of such medicinal effects such as anti-lipaemic, blood-hypocholesterolaemic, and anti-tumoral etc.

The protein quality and digestibility of algae have not yet been elucidated in contrast to that of other higher plants. We have already succeeded in extracting, with dilute alkali solution, major proteins from algae with relatively high protein values (ARASAKI and MINO 1973).

These experiments were fundamental studies on the value of algal proteins as a food source. In this paper, emphasis will be placed on the digestibility of algal proteins extracted from eight species of edible marine algae in Japan, namely, *Codium fragile, Ulva pertusa, Eisenia bicyclis, Undaria pinnatifida, Laminaria japonica, Analipus japonicus, Grateloupia turuturu,* and *Porphyra tenera.* The proteolytic enzymes used were pepsin,

pancreatin and a bacterial protease, pronase.

Materials and Methods

1. Materials: Fronds were collected along the coast of the central part of Japan, washed with water, and then frozen and stored at −20°C.

2. Preparation of proteins from algae: The preparation of the alkali soluble protein was carried out with dilute alkali solution as reported previously (ARASAKI and MINO 1973).

Frozen fronds were pretreated with acetone and ether-methanol mixture (1 : 1) and further extracted with 5% NaOH. The proteins were precipitated by adjusting the pH of the solution to 4.0 with acetic acid. This procedure was repeated three times, and the purified protein, as a pale, greenish-white powder, was isolated.

3. Pulverized algae: Frozen algae were dried at 105-110°C and then pulverized for the later experiments described below.

4. *In vitro* digestion: The *in vitro* digestion study of alkali soluble proteins was performed by the method reported previously (ARASAKI and MINO 1976).

Table 1. Amino acid compositions of the alkali soluble proteins in the various marine algae*.

(g of amino acid-N/100 g of protein-N)

Amino acid	Green		Brown				Red		
	Ulva pertusa	*Codium fragile*	*Eisenia bicyclis*	*Undaria pinnatifida*	*Analipus japonicus*	*Laminaria japonica*	*Porphyra tenera*	*Grateloupia turuturu*	*Ovalbumin***
Trp	0.3	1.2	1.3	0.8	1.6	0.6	1.3	0.7	1.0
Lys	4.5	4.1	7.8	4.3	6.9	6.6	4.5	4.3	7.7
His	4.0	1.5	4.0	2.7	3.3	3.9	1.4	1.8	4.1
NH_3	1.9	3.4	3.2	2.5	3.1	3.1	5.1	1.9	5.3
Arg	14.9	12.3	18.6	7.5	12.5	9.4	16.4	15.8	11.7
Asp	6.5	6.4	5.0	5.6	6.1	6.6	7.0	5.7	6.2
Thr	3.1	2.9	2.3	2.4	3.0	3.7	4.0	3.0	3.0
Ser	3.0	2.8	2.3	2.8	3.1	3.6	2.9	2.8	6.8
Glu	6.9	6.1	7.6	5.1	6.0	5.7	7.2	6.3	9.9
Pro	4.0	3.6	4.5	2.8	3.4	3.3	6.4	5.1	2.8
Gly	5.2	5.1	6.5	4.4	5.2	6.2	7.2	5.0	3.4
Ala	6.1	6.6	7.0	4.8	5.9	7.5	7.4	5.5	6.7
**Cys	1.2	0.6	0.7	0.5	1.1	1.9	0.3	0.7	1.4
Val	4.9	5.8	5.9	4.1	4.5	5.1	6.4	4.9	5.4
Met	1.6	2.0	1.7	2.0	1.6	1.4	1.1	2.0	3.1
Ile	3.5	3.4	4.4	2.9	3.2	3.5	4.0	4.4	4.8
Leu	6.9	6.6	7.3	5.1	5.9	6.0	8.7	6.3	6.2
Tyr	1.4	1.2	2.1	1.6	1.9	1.6	2.4	0.9	1.8
Phe	3.9	3.3	4.0	3.7	3.2	3.8	3.9	3.7	4.1
Total	83.8	78.9	96.9	65.8	81.5	83.5	98.2	81.2	95.4
N %	13.2	13.7	10.6	11.6	11.7	10.4	13.6	14.4	15.8

* FUJIWARA-ARASAKI *et al.* (1984)

** Cys Performic acid oxidation

*** LARSEN and HAWKINS (1961)

The enzyme solutions, pepsin (1 : 10,000 pu, k/g, Nakarai Chemicals Ltd.) in N/50 HCl, pancreatin (Difco Laboratories) in M/50 phosphate buffer (pH 7.6), and pronase (45,000 pu, k/g, Riken Chemicals Co.) in M/20 tris buffer (pH 8.6), were used. Final enzyme and substrate concentrations were adjusted to 0.05-0.1% and 0.5-1%, respectively. The proteolytic digestion of milk casein (Hammersten, Merck), under the same conditions described above, was used as control. The reaction mixture (enzyme and substrate, 1 : 1) was incubated at 37°C for 0, 0.5, 2, 3, 4, 5 and 24 hours. To each 1 m*l* of the reaction mixture was then added 4 m*l* of 5% trichloroacetic acid (TCA) to stop the reaction. The mixture was then allowed to stand at room temperature for 30 min. The optical density of the filtrate was measured by a spectrophotometer (Shimadzu Model UV-180) at 280 nm.

5. Five and twenty-four hours' digestibility: The filtrate (1.0 m*l*) of the reaction mixture (hydrolysed for eight hours with 1.0 m*l* of conc. H_2SO_4) was analysed to determine the nitrogen content by the Micro-Kjeldahl method (ARASAKI *et al.* 1979). The resultant digestibility was expressed in terms of percentage based on the comparable casein digestibility.

Results and Discussion

1. Alkali soluble proteins: The nitrogen

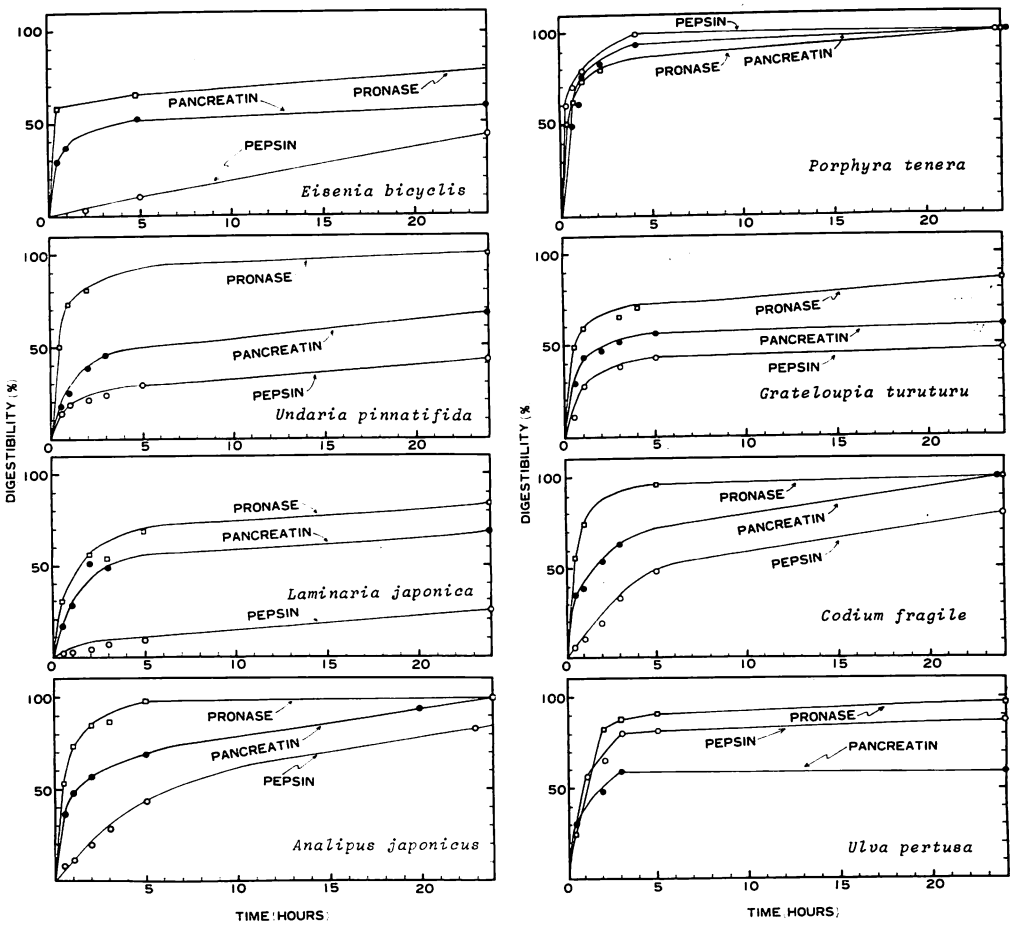

Fig. 1. Digestion curves of alkali soluble proteins with three enzymes, pepsin, pancreatin and pronase. Values of the digestibility is expressed as a percentage of casein digestibility.

content of the proteins were found to be 10-14%, which is somewhat lower than that of common proteins. The eighteen kinds of amino acids were detected in the protein and the recovery of amino acid nitrogen obtained in these experiments were of the order of 65-98% as shown in Table 1. In general, the essential amino acid content of the alkali soluble proteins was found to be present at relatively high levels, except for lysine, threonine and sulfur-containing amino acids. The amino acid compositions of the proteins in eight species were found to be similar and these results agreed well with the data obtained by LARSEN and HAWKINS (1961) from the two brown algae.

2. *In vitro* digestibility of the algal proteins: *In vitro* digestibility of the alkali soluble proteins obtained from eight species of algae was examined using three enzymes, pepsin, pancreatin and pronase. The proteolytic digestion of algal proteins was compared with that of milk casein under the same conditions described above. The percent digestion calculated proportionally against milk casein digestibility, (which was regarded as 100%), was plotted against time as shown in Fig. 1.

It was shown that the approximate maximum levels of digestion of the eight species of algae occurred after four to five hours and the digestion level then slowly increased during the next twenty-four hours. Of the three enzymes, pronase (bacterial enzyme), was shown to produce the highest level of digestion, and the next highest was pancreatin. The lowest digestion was induced by pepsin.

The results of five and twenty-four hours' digestibility experiments, performed by the Kjeldahl method (ARASAKI *et al.* 1979), where the nitrogen content in the filtrates of the reaction mixture was determined, are shown in Tables 2 and 3, respectively. The digestibility values were expressed as a percentage of digestion of casein digestibility, which was regarded as 100%. As shown in Table 2, five hours' digestion with pepsin was found to be lower (15-56%) than those with the two other enzymes, pancreatin (37-68%) and pronase (59-94%). The digestibility with pancreatin of three types of algae, *Ulva pertusa*, *Analipus japonicus* and *Porphyra*

Table 2. *In vitro* digestibility for five hours of alkali soluble proteins of algae*

Algae	Pepsin (%)	Pancreatin (%)	Pronase (%)
Codium fragile	22.5	37.2	70.9
Ulva pertusa	17.0	66.6	94.8
Eisenia bicyclis	18.7	53.7	59.1
Laminaria japonica	39.0	54.0	83.9
Undaria pinnatifida	23.9	48.1	87.2
Analipus japonicus	42.7	68.3	97.8
Grateloupia turuturu	15.8	34.0	59.2
Porphyra tenera	56.7	56.1	78.4

* Five hours' digestion: Nitrogen in the filtrates of the reaction mixture was determined by the Kjeldahl method as an estimate of digestion. The digestibility is expressed as a percentage based on the digestibility of casein. Casein digestibility was shown to be 64.3%, 90.1% and 92.9% by pepsin, pancreatin and pronase digestion, respectively, measured after five hours.

Table 3. *In vitro* digestibility for twenty-four hours of alkali soluble proteins of algae*

Algae	Pepsin (%)	Pancreatin (%)	Pronase (%)
Codium fragile	80.4	100.0	100.0
Ulva pertusa	86.2	66.2	96.2
Eisenia bicyclis	43.4	60.2	81.0
Laminaria japonica	48.5	71.2	86.3
Undaria pinnatifida	42.4	68.0	90.7
Analipus japonicus	85.0	100.0	100.0
Grateloupia turuturu	46.6	66.6	88.5
Porphyra tenera	90.9	70.5	98.3

* Twenty-four hours' digestion: Nitrogen in the filtrates of the reaction mixture was determined by the Kjeldahl method as an estimate of digestion. The digestibility is expressed as a percentage based on the digestibility of casein. Casein digestibility was shown to be 68.6%, 100.9% and 100.6% by pepsin, pancreatin and pronase digestion, respectively, measured after twenty-four hours.

tenera, was found to be 66.6, 68.3, and 56.1%, respectively, which was appreciably higher than those of the other species. Digestion with pronase was, however, found to be higher than those with the two other enzymes. The digestibility with pronase of two species, *Ulva pertusa* and *Analipus japonicus*, were also found to be 94.8 and 97.8% respectively, at the highest level. Normal digestibility with pronase was found to be about 70% in other species except in *Grateloupia turuturu* and *Eisenia bicyclis* (Table 2). As shown in Table 3, twenty-four hours' digestibility of algal proteins was found to be 42.4-90.9% with pepsin, 60.2-100% with pancreatin and 81.0-100% with pronase, and was higher than those after five hours' digestion. In particular, the digestibility with pancreatin of *Codium fragile* and *Analipus japonicus* was as high as that of casein. Six other species were also found to give the values of about 40 to 60% based on casein digestibility. Digestibility with pepsin, however, was as low as 43.4%, 48.5%, 42.4% and 46.6% in *Eisenia bicyclis*, *Laminaria japonica*, *Undaria pinnatifida* and *Grateloupia turuturu*, respectively. Digestibility of algae by pronase was found to be highest when compared with those by others enzymes. However, *Eisenia bicyclis* (81.0%), *Laminaria japonica* (86.3%) and *Grateloupia turuturu* (88.5%) were incompletely digested even after twenty-four hours' digestion.

In animal feed studies on dried algal meals some cases of low digestibility have been reported by previous investigators (BENDER *et al.* 1953, KIMURA 1952, MATSUKI 1960, and MORI *et al.* 1948).

KIMURA (1952) reported that the protein availability was 57.0% in *Laminaria japonica*, 64.1% in *Undaria pinnatifida* and 72.6% in *Porphyra tenera*, and MATSUKI (1960) also reported that it was 16.4% in *Laminaria*, 44.1% in *Undaria*, 70.8% in *Porphyra* and 44.9% in *Hijikia fusiforme* in human diets. According to MATSUKI (1960), the protein digestibility of algae was found to be higher than that of fungi and somewhat lower than that of the leaf portion. MORI *et al.* (1948) reported that the protein digestibility of eleven species of marine algae was low (15.1-71.5%). LARSEN and HAWKINS (1961) reported that values as high as 75% often observed with egg albumin could be observed in feeding tests for rats, using protein extracted with 20% sodium carbonate from two species of algae, *Chondrus crispus* and *Laminaria digitata*. Previous investigators reported that, in general, the lower digestibility of algal proteins was observed using raw materials (BENDER *et al.* 1953, KIMURA 1952, MATSUKI 1960, MORI *et al.* 1948).

In an attempt to clarify the low digestibility of the raw materials, we carried out on *in vitro* digestion of proteins by comparing the pulverized algae with the extracted proteins using *Porphyra tenera* and *Grateloupia turuturu*. The results showed without doubt that the digestibility of the pulverized algae was very low (Table 4).

In vivo experiments, however, the digestibility of *Porphyra tenera* in the raw materials was found to be 70.8% by MATSUKI (1960), 72.6% by KIMURA (1952) and 54.3-71.5% by MORI *et al.* (1948) where it was found to be higher than that of the other species tested. The result of the *in vitro* digestibility of extracted protein of *Porphyra tenera* obtained in the present experiment agreed well with the results of these investigators mentioned above. Hence, it appears that the protein

Table 4. Digestibility of pulverized algae, *Porphyra tenera* and *Grateloupia turuturu**

Algae	Enzyme	Digestibility (%)
Porphyra tenera	Pepsin	1.6
	Pancreatin	0.7
	Pronase	4.7
Grateloupia turuturu	Pepsin	6.1
	Pancreatin	9.1
	Pronase	18.6

* The digestibility is expressed as a percentage based on casein digestion. The casein digestibility was determined as 63.0%, 89.1% and 97.8% in pepsin, pancreatin and pronase digestions, respectively, measured after five hours.

in *Porphyra tenera* was easily solublized from the cell than in the case of the other species.

Recently, the similarity of protein digestibility of mono-cellular algae, *Chlorella*, and blue-green algae, *Nostoc muscorm* to human food has received attention and studies were also conducted to investigate their digestibility (COOK 1962, SUBBA *et al.* 1972, MITSUDA *et al.* 1977 and ISHII *et al.* 1974).

MITSUDA *et al.* (1977 a, b) reported that the *in vitro* digestibility in *Nostoc muscorm* is 74.4% with pepsin and 63.8% with trypsin and that of *Chlorella* with trypsin is 44.6% for dried cell and 70.9% for broken cells. MITSUDA *et al.* (1977b) also reported that the *in vivo* digestibility in *Chlorella* by rats is 59.7% for dried cells and 79.5% for broken cells (by Dyno-Mill), respectively. It was concluded by these authors that broken cells offer a good substrate for the utilization of *Chlorella* protein.

ISHII *et al.* (1974) also reported that the protein digestibility of *Chlorella* with trypsin was 69.3% for frozen cells and 86.1% for extracted protein. These results were similar to those of animal tests described above.

COOK (1962) reported that the protein digestibility of *Chlorella* by rat *in vivo* was 65.4% for dried cells and 73.0% for cells heated at 100°C for 30 min.

According to both IGARASHI *et al.* (1978) and ISHIHARA *et al.* (1968), carbohydrates and tannins i. e. phenolic compounds contained as impurities in the algae inhibited the protein digestion *in vivo*, and the digestion of *Chlorella* proteins with trypsin was inhibited not only by lipids but also by some pigments present in *Chlorella*. The alkali soluble proteins obtained in the present experiment were contaminated with small amounts carbohydrates and pigments. In view of the results from *Chrorella* the inhibition of protein digestion obtained in the present work may be due to contaminating substances.

In conclusion, the digestibility of eight species of Japanese edible marine algae used in the present experiment was found to be lower after five hours' digestion than those of animal proteins, most of them being as low as 50% of casein. However, the percentages of *in vitro* digestibility by emzymes could be improved to give the values of 42.4 to 90.9% with pepsin and to those of 60.2 to 100% with pancreatin, respectively, over twenty-four hours' digestion. This observation suggests that algal proteins may be a good source of human nutrition by further improvement of the protein digestibility.

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新崎輝子：海藻タンパク質の消化性

本研究は食糧資源としての海藻の基礎研究として，8種の日本産海藻について，著者の方法により分離したアルカリ可溶タンパク質を用い，ペプシン (1)，パンクレアチン (2)，プロナーゼ (3) のタンパク分解酵素による経時的人工消化を，ミルクカゼインを対照として行った。

その結果5時間消化では，(1) は 15～56%，(2) は 37～68%，(3) は15～56% でやや低い分解率を得たが，24時間では (1) は 42～91%，(2) は 60～100%，(3) は 81～100% でかなりよく，海藻によってはカゼインと同程度のものもあった。比較のために藻体の粉末物を用いて同様の消化実験を行ったが，その結果は非常に悪く，抽出タンパク質がすぐれていることを示した。(560 神戸市中央区諏訪山町 3-1　神戸山手女子短期大学)