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Nitrogen Conversion Factors and *in vitro* Protein Digestibility of some Seaweeds

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數種海藻의 蛋白系數와 *in vitro* Digestibility

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海藻의 營養學的인 基礎資料를 얻기 위하여 multi-enzyme system을 利用한 체외소화율과 아미노산분석을 기초로 한 蛋白系數를 測定하였다.

김 (*P. tenera*)의 체외소화율은 78.5~82.2로서 産地와 건조조건에 따라 약간의 차이를 보였으며, 잎파래(*E. linza*)나 다른 갈조류 (미역 *U. pinnatifida*, 톳 *H. fusiforme*, 모자반 *S. fuvellum*)에 비하여 높았고 효소활성저해물질 (trypsin inhibitor)의 함량은 갈조류에서 높았다. 잎파래는 체외소화율이 김보다 낮음에도 불구하고 효소활성저해물질이 가장 낮은 특이한 결과를 보였다. 전체적으로 해조류의 체외소화율이 다른 연구자들의 生體實驗에 의한 소화율 (*in vivo* digestibility)보다 높은 결과를 보인 것은 multi-enzyme system을 이용한 체외소화율 측정 방법을 해조류의 정확한 소화율 측정에 적용하기에는 문제성이 있는 것으로 밝혀졌다.

日乾 김에 대한 microwave cooking의 영향은 가열시간이 15분 경과하여도 현저한 소화율 증가는 볼 수 없었으며, 효소활성 저해물질함량은 서서히 감소하는 경향을 보였다. 또한 한국식으로 구운김의 체외소화율은 microwave로 15분 가열한 시료와 비슷하였다.

아미노산 분석 결과를 이용한 단백계수(Factor method)는 김의 경우 6.52로 계산되었고, 잎파래는 6.00, 미역 6.11, 모자반 5.85, 톳은 5.83이었으며, Kjeldahl 질소분석결과를 이용한 단백계수(Kjeldahl Method)는 김 6.29, 잎파래 5.83, 미역 5.40, 모자반 5.45, 톳 5.49로 나타나, 종래의 粗蛋白系數 (6.25)보다 낮은 결과를 보였다. 해조中의 非蛋白態窒素의 정확한 규명이 없는 상태에서, 해조의 단백질함량 측정에는 아미노산 분석결과를 이용한 새로운 단백계수(Factor Method)를 사용함이 바람직한 것으로 생각되었다.

Introduction

Nitrogen in foods not only comes from amino acids in protein but also exists in additional forms that may or may not be used as a part of the total nitrogen economy of humans and animals (Pellet *et al.* 1980). The nitrogen content of proteins in foods can be various, depending on the amino acids they comprise. In addition, purines, pyrimidines, free amino acids vitamins, creatine, creatinine, and amino sugars can also contribute to the total nitrogen

present. In meat a portion of the nitrogen occurs as free amino acids and peptides; fish may contain these, volatile-basic nitrogen and methyl-amino compounds. Marine elasmobranchs may also contain urea. Half of the nitrogen of the potato may not be in the form of protein (Neubeger and Sanger, 1942) and even in human milk as much as 50 percent of the total nitrogen may be urea nitrogen (Erickson *et al*, 1963).

Because the nutritional significance of much of the non-amino acid and non-peptide nitrogen is unclear, nitrogen analysis of a food is usually much more precise than the nutritional significance that can be attached to it.

Generally, in discussion of the nutritional potentiality and quality of seaweeds as foodstuffs, the quantity of crude protein, included non-protein nitrogen, which was calculated from the amount of nitrogen determined by the Kjeldahl method, in these materials has been accentuated. And the nitrogen content is customarily multiplied by a factor of 6.25 to calculate the crude protein content.

The 6.25 factor is used for most feed materials; the practice apparently originated from early research on proteins of animal origin which were found to contain approximately 16% nitrogen (100/16=6.25). The practice of using 6.25 as a factor in calculating protein content is, however, based on an incorrect assumption and a number of erroneous conclusions as mentioned above. That this assumption was incorrect was recognized in 1931 by D.B. Jones, who calculated more accurate nitrogen-to-protein factors by taking into account the fact that different-plant proteins contain various amounts of nitrogen (Jones 1931).

Therefore, it is considered that determimination of protein content in seaweeds is more important than the crude protein content showed in numerous investigation since Oya (1935), in order to evaluate the nutritional quality of seaweed. This work was undertaken to determine accurate protein value of seaweed using quantitative amino acid data on the basis of nitrogen-to-protein factors for seaweeds, applying the approaches made by Tkachuk *et al.* (1969) and Morr (1981).

On the other hand, it was known that the digestibility of seaweed is lower than that of other plant proteins and it has been considered as a main factor which influenced on the nutritional value of seaweed. It is assumed that the lower digestibility was resulted by the complicate factors such as protein content of nitrogenous constituent, thoughness (structual characteristics) and constituents of its cellulosic cell wall. A number of authors have commented on the nutritional value of seaweed predicted as EAAI (Larsen and Hawkins 1961), or PPD index (Woo *et al*. 1978). The present study was conducted to determine the *in vitro* digestibility of seaweeds using the multienzyme-automated assay developed by Satterlee et al. (1979).

Materials and Methods

1. Collection and preparation of samples

Samples used in the present study were *Porpyra tenera* (red seaweed), *Enteromorpha linza* (green seaweed), and three kinds of brown seaweed (*Undaria pinnatifida*, *Hizikia fusiforme*, *Sargassum fulvellum*). They were collected from Dadaepo (*P. tenera* and *E. linza*) and Yangsan (brown seaweeds) near Busan city on April 16, 1982, and freeze dried by using SINKU KIKO ULVAC freeze dryer for 24 hours at 0.08 mmHg and a plate temperature of 40°C. After freeze drying, samples were ground in micromill (JANK & KUKEL, IKAWERK Type AIOSI, v/min. 20,000), adding some dryice piecies in order to avoid thermal denaturation of proteins, to pass a 100-mesh screen of standard sieve. In order to check the effect of heat treatment on *in vitro* protein digestibility, commercially sundried laver (*P. tenera*) were obtained from Jindo, Korea. It were heated on hot plate as Korean traditional recipe for periods from 30 seconds to 15 minutes before grinding.

2. Nitrogen and *in vitro* protein digestibility assay

Nitrogen was determined by the Kjeldahl method (AOAC, 1980). The *in vitro* protein digestibility was measured for both sundried and freeze dried seaweed samples using a multienzyme automatic recording techniques described in AOAC (1982).

3. Trypsin inhibitor content

Trypsin inhibitor content of seaweed samples was determined using the procedure of Hamerstrand *et al.* (1981).

4. Amino acid analysis

Amino acid composition of the samples was determined using a Beckman 120C amino acid analyzer. The samples were hydrolyzed with 6 N HCI, under vacuum, for 24 hours at 110°C to release the acidic, neutral and basic amino acids. Tryptophan was released using alkaline hydrolysis (Hugli and Moor, 1972), the sulfur-containing amino acids were quantitatively released using a performic acid pre-treatment of the samples followed by a 6 N HCI hydrolysis (Moor, 1963).

5. Calculation of nitrogen conversion factor

The first method (Factor Method) involved multiplying the quantity of each amino acid by its molecular nitrogen factor. The resulting weighted nitrogen values were summed to provide a more precise amino acid nitrogen content of the protein, based on amino acid composition. The total amino acid content was then divided by this total amino acid nitrogen value to obtain a nitrogen conversion factor. The second method simply involved dividing the total amino acid content by the micro-Kjeldahl nitrogen value to obtain a nitrogen conversion factor.

Results and Discussion

1. *In vitro* protein digestibility of edible seaweed

In order to estimate the potential and properties of usable proteins, three kinds of seaweed (6 species) were selected and checked for *in vitro* digestibility and trypsin inhibitor content. The *in vitro* digestibility of seaweed as shown in Table 1 was higher than the *in vivo* results obtained by other researchers for seaweed or algal protein (Larsen and Hawkins, 1961; Clement *et al.*, 1967; Lipinsky and Litchfield, 1974; Kang, 1976; Yu *et al.*, 1975; Devi *et al.*, 1981; Barta *et al.*, 1981). The significant difference in the digestibility between *in vivo* and *in vitro* assays indicates that there are some problems in predicting the digestibility of seaweed using multienzyme technique of Satterlee *et al.* (1979). It was so difficult to adjust pH of digestion solution and checking the changes of pH during digestion period because of the viscosity of extracted polysaccharides from ground seaweed samples at alkali conditions (pH 8.0). This was especially a problem for red and brown seaweeds. Therefore, it is assumed that the poorest *in vitro* digestibility of brown seaweed was a result of complicating factors such as protein content of nitrogenous constituents, and thoughness (structural characteristics) and constituents of its cellulosic cell walls. The problem of protein in brown seaweed can be seen in the data reported by Larsen and Hawkins (1961) that the highest EAAI was shown in red seaweed and lowest in brown seaweed. This tendency was also given in the results obtained by Woo *et al.* (1978) that PPD index of red seaweed was 69 (*P. tenera*) while the poorest(25) was revealed in brown seaweed (*S. fulvellum*). On the other hand, it was thought that the algal pigments including the so-called biliproteins such as phycoerythrins and phycocyanins which are covalently bound to protein mentioned by some

Table 1. Comparison of the *in vitro* protein digestibilitiy and trypsin inhibitor contents of sun dried laver (*Porphyra tenera*) and other seaweeds of Korea

Sample	Species	Origin of sample	Product description	Nitrogen (%)	*In vitro* digestibility (%)	Trypsin inhibitor (mg/g)
Laver	*Porphyra tenera*	Kimhae	Sun dried	6.78	82.2	0.31
Laver	*Porphyra tenera*	Jindo	Sun dried	5.83	79.9	0.26
Wild laver	*Porpyra suborbiculata*	Busan	Freeze dried	5.37	78.5	0.35
Green alga	*Enteromorpha linza*	Busan	Freeze dried	5.65	78.5	0.12
Gulf weed	*Sargassum fuvellum*	Yangsan	Freeze dried	3.01	73.4	0.33
Sea mustard	*Undaria pinnatifida*	Wando	Blanched then sun dried	2.99	80.2	0.33
Sea mustard	*Undaria pinnatifida*	Yangsan	Freeze dried	2.94	77.1	0.55
Fusiforme	*Hizikia fusiforme*	Yangsan	Freeze dried	1.17	72.0	0.54
Bull kelp	*Nereocystis leutkena**		Freeze dried	2.44	79.6	

* Data from Barta E.S. *et al.* (1981)

Table 2. Influence of microwave and hot plate heating upon the *in vitro* protein digestibility of sun dried laver (*Porphyra tenera*)

	Raw	Microwave heating time (min).						Hot plate[a]
		0.5	1	2.5	5	10	15	
In vitro digestibility (%)	79.9	81.1	81.1	82.0	82.6	83.5	84.3	84.5
Trypsin inhibitor[b] (mg/g)	0.26	0.28	0.26	0.26	0.16	0.15	0.13	0.04

a Korean traditional cooking method
b Hamerstrand method (1981)

authors (OhEocha, 1966; Kim and Nam-Kung, 1976) influenced on the *in vitro* protein digestibility of red seaweed also and a chlorophyll lipoprotein complex occurring in the green seaweed's chloroplast fraction resisted *in vivo* as well as *in vitro* digestion (Arai, 1981). As is shown in Table 1, the trypsin inhibitor content of green seaweed (*E. linza*) was lowest of all seaweed samples, while brown seaweed which contained a high of polysaccharides (Zajic, 1970) showed the highest value in trypsin inhibitor content.

2. Variation of *in vitro* digestion and trypsin inhibitor content after microwave cooking

In an attempt to obtain a cooked seaweed with highest possible digestibility, microwave and hot-plate heating were tried for the sundried laver (*P. tenera*). The compartive values for *in vitro* digestibility and trypsin inhibitor content are shown in table 2. The *in vitro* digestibility of sun dried laver was increased with cooking time, although it was not so significant. The sample of hot plate heating showed increased *in vitro* digestibility about 5%, while the trypsin inhibitor content was lowered to 15% of that of a non-heated sample. This suggested that cell wall were ruptured during heating, and cell contents then became more susceptible to enzyme digestion.

3. Amino acid profiles of seaweed

As shown in Tables 3 and 4, the amino acid contents of seaweed were expressed as averages of three replicate determinations. Generally, the amino acid compositions given in the present work agree quite well with few available reports (Mazur and Clarke, 1938; Smith and Young 1955; Kim, 1974; Yu *et al.*, 1975; Woo *et al.*, 1979; Narasimha *et al.*, 1980). In most of the latter, results were obtained by

Table 3. Amino acid profiles of *Porphyra tenera* and *Enteromorpha linza* [a]

Amino acid	*Porphyra tenera*			*Enteromorpha linza*		
	g/16gN	%	x M.N.F. [b]	g/16g N	%	x M.N.F.
Asp	9.77	4.01	0.422	9.22	3.26	0.343
Thr	5.79	2.38	0.280	4.20	1.48	0.174
Ser	5.52	2.27	0.302	3.73	1.32	0.176
Glu	11.40	4.68	0.445	12.64	4.47	0.426
Pro	4.39	1.80	0.219	4.10	1.45	0.177
Gly	6.84	2.81	0.524	7.43	2.63	0.491
Ala	12.12	4.97	0.783	8.20	2.90	0.456
Val	5.89	2.42	0.289	4.76	1.68	0.201
Met	2.62	1.08	0.102	2.58	0.91	0.086
Ile	3.57	1.46	0.157	4.10	1.45	0.155
Leu	7.48	3.07	0.328	6.23	2.20	0.235
Tyr	3.39	1.39	0.108	2.87	1.01	0.078
Phe	4.20	1.72	0.240	4.75	1.68	0.143
Lys	5.43	2.23	0.428	4.15	1.47	0.282
His	1.34	0.55	0.148	1.49	0.53	0.144
Amm	1.75	0.72	0.595	3.42	1.21	0.996
Arg	6.24	2.56	0.823	7.47	2.64	0.850
Cys	1.86	0.76	0.088	1.04	0.37	0.043
Trp	1.00	0.41	0.506	0.88	0.31	0.043
Total	100.60	41.29	6.337	93.26	32.97	5.496

a Average of three replicate determinations
b Molecular nitrogen factor

Table 4. Amino acid profiles of brown seaweed [a]

Amino acid	*Undaria pinnatifida*			*Sargassum fuvellum*			*Hizikia fusiforme*		
	g/16g N	%	x M.N.F. [b]	g/16/g N	%	x M.N.F.	g/16g N	%	x M.N.F.
Asp	9.28	1.17	0.180	11.45	2.13	0.225	12.22	0.90	0.094
Thr	4.53	0.83	0.098	5.50	1.03	0.121	4.04	0.30	0.035
Ser	4.37	0.80	0.107	4.37	0.81	0.109	3.84	0.28	0.038
Glu	13.76	2.53	0.241	11.96	2.23	0.211	9.72	0.71	0.068
Pro	3.26	0.60	0.073	3.02	0.56	0.069	3.49	0.26	0.031
Gly	4.29	0.79	0.147	4.28	0.80	0.149	4.86	0.36	0.067
Ala	4.85	0.89	0.140	4.70	0.88	0.138	6.40	0.47	0.074
Val	5.28	0.97	0.116	4.76	0.89	0.106	5.04	0.37	0.044
Met	2.47	0.45	0.042	1.87	0.35	0.033	2.12	0.16	0.015
Ile	3.38	0.71	0.076	3.38	0.63	0.067	4.90	0.36	0.038
Leu	8.48	1.56	0.167	8.00	1.49	0.159	7.20	0.53	0.056
Tyr	2.66	0.49	0.038	2.62	0.49	0.038	2.41	0.18	0.014
Phe	3.77	0.69	0.059	5.06	0.94	0.080	0.09	0.37	0.032
Lys	4.69	0.86	0.165	3.58	0.67	0.128	3.88	0.28	0.055
His	1.48	0.27	0.073	1.06	0.20	0.054	0.65	0.05	0.009
Amm	4.15	0.76	0.626	4.93	0.92	0.757	4.98	0.37	0.301
Arg	3.57	0.67	0.216	4.78	0.89	0.287	4.96	0.36	0.117
Cys	0.93	0.17	0.020	1.01	0.19	0.022	1.00	0.07	0.008
Trp	0.87	0.16	0.022	0.86	0.16	0.022	0.75	0.06	0.008
Total	86.57	15.91	2.606	87.19	16.25	2.776	87.58	6.44	1.104

a Average of three determinations
b Molecular nitrogen factor

ion-exchange chromatographic procedures. However, comparisons between present results and the majority of compositions listed in the literature reveals numerous and wide discrepancies. These should not be interpreted too strictly, since compositions are being compared different samples grown in different environments and locations as described by Ogino (1951). The amount of tryptophan in *P. tenera* and *E. linza* was smaller than in the results of Woo *et el.* (1979) using colorimetric assay of Spies and Chamber (1948) and showed the larger amounts in brown seaweed. This was due to the higher contents of polysaccharide in brown seaweed resulting the raised recovery of tryptophan, as described by Hugli and Moor (1972). Recoveries of cystine, methionine, threonine, and serine were higher than those in most literature reports. Higher recoveries of cystine probably resulted from analyzing it in more stable form of cysteic acid. The higher yields of threonine and serine might be due to correcting for decomposition which occurs during hydrolysis. The higher recovery of methionine was possibly due to using thiodiglycol as an antioxidant, as recommended by Moor and Stein (1951).

4. Nitrogen conversion factors of seaweed proteins

It is seen from Table 5 that the conversion factor (Factor Method) of seaweed from 6.52 (*P. tenera*) to 5.85 (*S. fulvellum*) and Kjeldahl conversion factors ranged from 6.29 (*P. tenera*) to 5.40 (*U. pinnatifida*). Differences in values of conversion factors must be related to the differences in amino acid compositions of seaweed sample. Higher relative amounts of ammonia, histidine, leucine and isoleucine will lower the value of the conversion factor by the both methods, whereas higher relative amounts of tryptophan, cystine, lysine, tyrosine, methionine, comma proline will raise the value. The greatest differences in both conversion factor sets were obtained for brown seaweed, such variation may be due to the high nonprotein nitrogen (Ogino, 1951) content and it indicates that the differences were higher in foods which had a lower nitrogen content as mentioned by Heidelbaugh *et al.* (1975). This finding suggests that result from the use of "traditional" Kjeldahl nitrogen conversion factors of seaweed (6.25) tend to introduce the more error about 13% overestimation into the protein content of brown seaweed and 6.7% for green seaweed (*E. linza*) than the use of Kjeldahl conversion factor shown in Table 5. Therefore, the use of such "traditional" conversion factor of seaweeds for the purpose of evaluation or "nutritional labeling" may be questioned. But in case of laver, the Kjeldahl factor is more in line with the "traditional" Kjeldahl nitrogen factor. It may be indicated that the amounts of nonprotein nitrogen in laver (*P. tenera*) is not significant. On the other hand, the nitrogen contents determined using Kjeldahl method were higher than the nitrogen contents derived from summation of nitrogen in each amino acid. However, the factor based on amino acid comp-

Table 5. Nitrogen conversion factors for experimental seaweeds

Sample	Total amino acid content	Amino acid N. content (% dry basis)	Kjeldahl N. content (% dry basis)	N. conversion factor	
				Factor method	Kjeldahl method
Porpyra tenera	41.29 [a]	6.337	6.565 [b]	6.52	6.29
Enteromorpha linza	32.97	5.495	5.655	6.00	5.83
Undaria pinnatifida	15.19	2.606	2.947	6.11	5.40
Sargassum fulvellum	16.25	2.776	2.983	5.85	5.45
Hizikia fusiforme	6.44	1.104	1.174	5.83	5.49

a Three replicate determinations
b Four replicate determinations

osition (Factor Method) would be expected as to be as accurate as using more rigorous values derived from analysis of the seaweed for each individual amino acid. An additional and most important advantages to using the Factor Method is that the amino acid compositional data would enable the food processor to determine the limiting amino acid and chemical score of the seaweed protein as indicators of its nutritional quality.

Summary

In an attempt to evaluate the nutritional quality of seaweed protein, the effects of heat treatment on the *in vitro* digestibility and trypsin inhibitor content in seaweed were determined. In this study, the nitrogen-to-protein conversion factors were also calculated on the basis of quantitative amino acid data. The results are as follows :

1. The *in vitro* protein digestbilty of red seaweeds (*P. teoera* anc *P. suborbiculata*) were ranged from 78.5 to 82.2, and green seaweed (*E. linza*) and brown seaweeds showed value under 80 *in vitro* digestibility. In general, trypsin inhibitor contents in brown seaweed were higher (0.33-0.54 mg/g) than those of red seaweeds (0.26-0.39 mg/g). And it is noted that the lowest trypsin inhibitor content was shown in green seaweed (*E. linza*) in spite of lowest *in vitro* digestibility (78.5).

2. The *in vitro* protein digestibility of sun dried laver (*P. tenera*) was increased with cooking time (microwave heating), but it was not significant. Hot plate cooking raised the *in vitro* digestibility from 81.1 to 84.5. The influence of cooking time on trypsin inhibitor content was inversely proportional to *in vitro* digestibility.

3. Computed nitrogen factor, based on amino acid content (Factor method) and Kjeldahl nitrogen content (Kjeldahl method), were 5.83 (*H. fusiforme*)- 6.52 (*P. tenera*) as Factor method and 5.40 (*U. pinnatifida*)-6.29 (*P. tenera*) as Kjeldahl method. Individual value for each nitrogen conversion factor differed by species, especially in brown seaweeds. The best estimate of the protein content of seaweed can be calculated from multiplying the summed amino acid content by conversion factor (Factor method).

References

AOAC 1982. Calculated protein efficiency ratio (C-PER and DC-PER). Official first action. J. of AOAC, 65(2), 496.

Arai, S. 1981. Deterioration of food proteins by binding unwanted compounds such as flavors, lipids and pigments. in "Chemical Deterioration of proteins". J. R. Whitaker and M. Fujimaki edited, ACS, Washinton D. C., 195-209.

Barta, E. S., A. L. Branen and H. K. Leung. 1981. Nutritional analysis of Puget sound bull kelp (*Nereocytis leutkeana*). J. Food Sci., 43(5), 1543.

Clement, G., C. Giddery and R. Menzi. 1967. Amino acid composition and nutritive value of the alga, *Spirulina maxima*. J. Sci. Food Agric., 18. 497.

Devi, M. A., G. Subbulakshmi, K. M. Devi and L. V. Venkataraman. 1981. Studies on the proteins of mass-cultivated, blue-green alga (*Spiruliba pastensio*). J. Agric. Food Chem., 29, 522.

Erickson, B. N., M. Gulick, H. A. Hunscher, and I. G. Macy. 1953. "Human milk studies; The non-protein nitrogen constituents", J. Biol. Chem., 106, 145-159.

Hamerstrand, G. E., L. T. Black, and J. D. Glover. 1981. Trypsin inhibitors in soy products; Modification of the standard analytical procedure. Cereal Chem., 58(1) 42.

Heidelbaugh, N. D., C. S. Huber, J. F. Bennarczyk, M. C. Smith, P. C. Rambaut, and H. O. Wheeler. 1975. Comparision

of three methods for calculating protein content of foods. J. Agric. Food Chem., 23(4), 611-613.

Hugli, T. E. and S. Moor. 1972. On alkaline hydrolysis of tryptophan. J. Bio. Chem., 247(9), 2828.

Jones, D. B. 1931. Factors for converting percentages of nitrogen in foods and feeds into percentages of proteins. U. S. Dept. Agr., Circ. No. 183. Washington, D. C.

Kang, M. H. 1976. A study on the digestibility of Korean seaweed by animal experiment. Research of Food Nutrition(E-Hwa Univ.), 6, 29.

Kim, J. P. 1974. Development of protein utilization from inedible algae. I. Separation of crude protein from inedible algae and its amino acid pattern in crude protein. Korean J. Food Sci. Technol., 6(1), 17-23.

Kim, J. P. and Nam-Kung S. 1976. Isolation of chromoprotein and its amino acids composition in Korean laver. J. Food Sci. Technol., 8(3), 172-178.

Larsen, B. A. and W. W. Hawkins. 1961. Nutritional values as protein of some of the nitrogenous constituents of two marine algae, *Chondrus crispus* and *Laminaria digitata*. J. Sci. Food Agric., 12, 523.

Lipinsky, E. S. and J. H. Litchfield. 1974. Single-cell protein in perspective. Food Tech., 28, 16-24.

Mazur, A. and H. T. Clarke. 1938. The amino acids of certainmarine algae. J. Biol. Chem., 123, 729.

Moore, S., ans W. H. Stein. 1951. Chromatography of amino acids on sulfonated polystyrene resins. J. Biol. Chem., 192, 663.

Moore, S. 1963. On the determination of cystine as cysteic acid. J. Biol. Chem., 238, 238.

Morr, C. V. 1981. Nitrogen conversion factors for several soybean protein products. J. Food Sci., 46, 1362-1367.

Narashima, D. L. R., G. S. Venkataraman, S. K. Duggal and B. O. Eggum. 1982. Nutritional quality of blue-green alga, *Spirulinaplatensis* CEITLER. J. Sci. Food Agric., 33, 456.

Neuberger, A. and F. Sanger. 1942. "The nitrogen of potato," Biochem, J., 36, 662-671.

Ogino, C. 1955. Biochmical studies on the nitrogen compounds of algae. J. of the Tokyo Univ. of Fish., 41(2), 107-155.

Oya, T., and K. Fujikawa. 1935. Kaiso no Kagaku., Tokyo, 1-17.

Pellett, P. L. and V. R. Young. 1980. Analytical methods for the determination of nitrogen and amino acids in foods, in "Nutritional Evaluaion of Protein Foods", The United Nations University, Tokyo, 7-25.

Satterlee, L. D., H. F. Marshall and J. M. Tennyson. 1979. Measuring protein quality. J. A. O. C. S., 56, 103.

Smith, D. C. and E. G. Young. 1955. The combined amino acids in several speceis of marine algae. J. Biol. Chem., 217, 845.

Spies, J. R. and D. C. Chamber. 1951. Spectrophotometric analysis of amino acid peptide with their salts. J. Biol. Chem., 191, 1781-1797.

Tkachuk, R. 1969. Nitrogen-to-protein conversion factors for cereal and oilseed meals. Cereal Sci., 46, 19-442.

Woo, S. I., H. S. Ryu and K. H. Lee. 1979. Studies on the extraction of seaweed proteins. 4. Precipitation conditions and nutritional evaluation of isolated seaweed proteins. Bull. of the Korean Fish. Soc., 12(4), 225.

Yi, J. Y., K. Y. Lee and S. H. Kim. 1975. A study on the nutritive value and utilization of powdered seaweeds. Korean J. Nutr., 8(1), 15.

Zajic, J. E. 1970. "Properties and Products of Algae", Plenum Press, New York-London, 1-22.