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Seasonal protein variation in the New Zealand seaweeds *Porphyra columbina* Mont. and *Porphyra subtumens* J. Ag. (Rhodophyceae)

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Seasonal variation in tissue protein was measured in the red algae *Porphyra columbina* Mont. and *Porphyra subtumens* J. Ag. by three methods (total nitrogen multiplied by 6.25, biuret protein, and sum of anhydroamino acids). The protein levels in both species showed a seasonal trend, with maximum levels occurring in the winter. The measured protein content varied between the methods used, with the nitrogen value multiplied by 6.25 giving the highest value, followed by the sum of anhydroamino acids and then the biuret method. The sum of amino acids appeared to be the most accurate method of determination. Based on the sum of anhydroamino acids, a new multiplication factor of 5.0 for the conversion of nitrogen to protein has been proposed. The predominant protein-bound amino acids were Ala, Glu, Asp and Leu, followed by Val, Lys and Arg. Ala was the main amino acid in *P. columbina*, but Glu was the main amino acid in *P. subtumens*. Similarly, Ala and Glu were the main free amino acids in *P. columbina* and *P. subtumens*, respectively. The protein-bound amino acids and the major free amino acids showed specific seasonality.

Key Index Words: amino acids—biuret—nitrogen—nori—Porphyra columbina—Porphyra subtumens—*protein—Rhodophyceae—seasonal.*

Mariculture of the red algae *Porphyra* and its processing into thin, purple-black sheets, called "hoshi nori", is a prominent food industry in Japan. *P. yezoensis* Ueda is now the main species used although *P. tenera* Kjellman was important in the past (Miura 1975; Nisizawa *et al.* 1987).

Porphyra is used as food in other parts of the world also. It is farmed in China, where it is known as "zicai" (Tseng 1981), and in Korea, where it is known as "kim" (Mumford and Miura 1984). It is consumed in smaller quantities in Wales and New Zealand, where it is known as "laver" and "karengo", respectively (Chapman and Chapman 1980). The most common species of *Porphyra* found in New Zealand are *P. columbina* Mont. and *P. subtumens* J. Ag. (Chapman 1969). *P. columbina*, a traditional food of the Maori, grows on rocky substrate in the intertidal zone on most of the coastline around New Zealand (Nelson 1984). Mariculture of *P. columbina* is being investigated (Brown *et al.* 1990). *P. subtumens* is an endemic epiphyte on *Durvillaea antarctica* (Chamisso) Hariot and *D. willana* Lindauer (Chapman 1969). Recent studies on New Zealand *Porphyra columbina* and *P. subtumens* include the determination of their ascorbic acid contents (Friedlander *et al.* 1989). The cell culture (Xue-Wu and Gordon 1987) and resistance to desiccation (Brown 1987) of *P. columbina* have also been investigated.

The protein content determined by different investigators for the same *Porphyra* species show considerable variation. Protein in *P. tenera* is reported to range from 14.9%, dry weight, (Mukai *et al.* 1981) to 56.1% (Noda and Horiguchi 1975). The only available

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literature on the protein content of *P. columbina* Mont. was 5.5%, dry weight, as reported by Quilhot (1970). Ji *et al.* (1981) studied the seasonal variation of amino acids in *P. yezoensis* in three locations differing in dissolved nitrogen content (30 μg to 300 μg NH_4-N/*l* seawater). The total amino acids ranged from approximately 10 to 30%, air dry weight. Lower protein contents occurred in regions with lower seawater nitrogen.

Several studies have been carried out on seasonal tissue nitrogen variation. Brown *et al.* (1990) found higher values in *P. columbina* Mont. (New Zealand) during the peak growth period (winter). The same trend was observed by Ji *et al.* (1981) in *P. yezoensis*, and by Takagi (1951) in several *Porphyra* spp.

Various methods have been used to study protein in *Porphyra*. Mukai *et al.* (1981) compared protein in the cell walls of *P. tenera* obtained by multiplying the nitrogen content by 6.25, summation of amino acids and a biuret-Folin method. They found the protein content calculated from nitrogen gave the same value as the sum of anhydroamino acids. This suggests that all nitrogen was present as amino acids, and the protein molecules contained 16% nitrogen. However, protein determined by the Lowry-biuret method gave considerably higher results than the other two methods. The nitrogen conversion factor 6.25 indicates 16% protein nitrogen, and assumes no non-protein nitrogen is present. This factor is applicable to egg, meat and legumes. The factor for refined flour is 5.70 and for milk and milk products is 6.38. The 6.25 conversion factor is often employed when the protein nitrogen content is not known. Protein calculated by this method should be referred to as "crude protein" (FAO/WHO 1973; FAO/WHO/UNU 1985). The nitrogen content of proteins can vary from 12 to 30% (Lillevik 1970). Arasaki and Mino (1973) found the nitrogen content of alkali soluble seaweed proteins, which they reported to be the major type of protein in *Porphyra*, ranged from approximately 12 to 14%. Determination of the protein nitrogen content usually involves extraction of the protein, and assumes the extraction is complete. However, Coulson (1955) and Smith and Young (1953) found polysaccharides can interfere with the extraction of protein from Phaeophyta. Three or four types of soluble proteins have been found in seaweeds (Takagi 1950; Arasaki and Mino 1973; Amano and Noda 1990). Seaweeds often contain non-protein nitrogenous compounds. These may include ammonia compounds, free amino acids, peptides, nitrates and pigments (Rosell and Srivastava 1985), and seaweeds produce non-protein amino acids (Impellizzeri *et al.* 1975; Fattorusso and Piattelli 1980).

Amino acids in algae may occur in combined (protein-bound) or free form (Young and Smith 1958). Ala, Glu and Asp have been found to be the predominant amino acids in *Porphyra* (Munda and Gubensek 1976; Ji *et al.* 1981; Amano and Noda 1990), while Harada *et al.* (1990) found Ala, Glu and Tau were the predominant free amino acids, with Asp also present. Tau has been found to be extensively distributed in the seaweeds of Rhodophyta (Impellizzeri *et al.* 1975; Fattorusso and Piattelli 1980). The biuret method, a commonly used colorimetric method, does not measure free amino acids or dipeptides. It is based on the solubility of protein in alkali, which varies for different proteins. Arasaki and Mino (1973) reported that *P. tenera* contained 65% alkali soluble protein.

The present study investigates the seasonal protein variation in *P. columbina* and *P. subtumens* measured by three methods, and looks at the possibility of using a conversion factor other than 6.25 for the nitrogen value. The study looks briefly at the quality of the protein present, and at the free amino acids, some of which have been reported to contribute to the flavour of nori.

Materials and Methods

Porphyra columbina was collected from rocks in the littoral zone of the rocky shore at St. Clair, Dunedin. *Porphyra subtumens*, an epiphyte on *Durvillaea antarctica* and *D. willana*,

was collected from the sublittoral zone at Brighton, 14 km south west of Dunedin. Collections of *P. subtumens* were restricted to periods of low tide (0.1 to 0.2 m below mean tide level). Whole plants were washed with seawater at the time of collection, to remove sand and epiphytes. Samples were pooled and drying at 30°C in an air circulating oven commenced within three hours of collection. Dried samples were ground in a Wiley Mill, to pass through mesh size 0.5 mm. The samples were then stored at room temperature (approximately 20°C) in air tight containers until analyzed. Each sample analyzed contained portions from at least 50 plants.

Total nitrogen was determined by the Dumas method, using a Coleman Model 29 Nitrogen Analyzer, and had an error of ±0.3%, dry weight. The measured nitrogen content was multiplied by 6.25 to obtain the "crude protein content". The biuret method (Goa 1953) as modified by Bergersen (1980) was used to determine the total protein content of the samples. Dialyzed bovine serum albumin (2.5 mg/ml distilled water) was used as a standard solution. Preliminary studies showed a green pigment, formed or unmasked by the addition of NaOH to the samples, caused interference. Consequently, determinations were carried out by simultaneously subtracting the pigment absorbance of samples that did not have Benedicts reagent added. Analyses were done in triplicate.

Amino acid analysis was used to determine the amino acids: aspartic acid, threonine, serine, glutamic acid, proline, glycine, alanine, valine, isoleucine, leucine, tyrosine, phenylalanine, histidine, lysine, arginine,

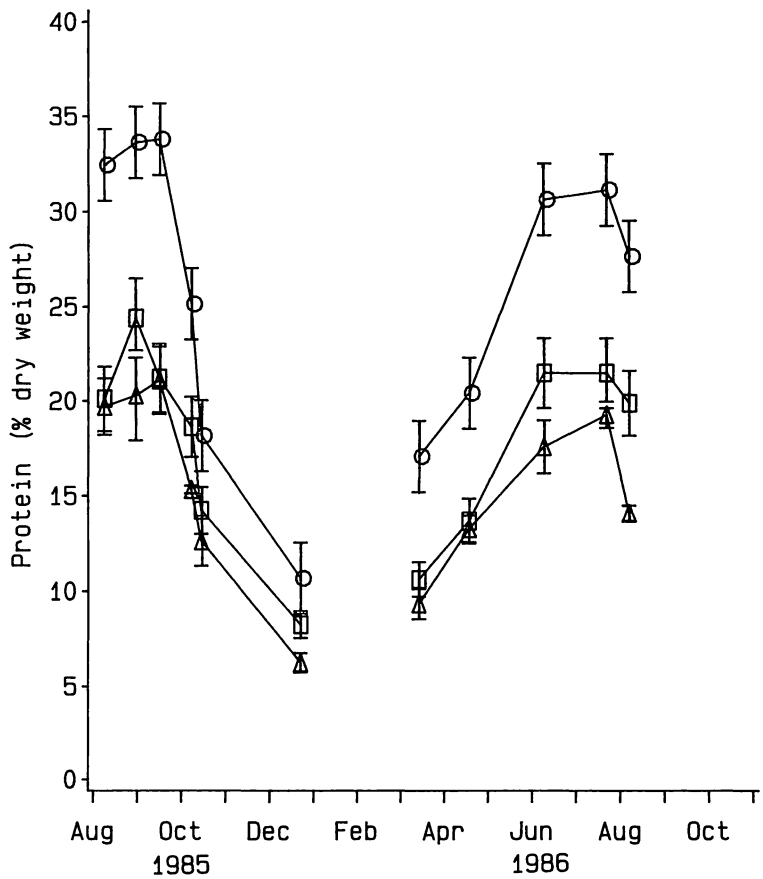

Fig. 1a. Seasonal variation in the protein content of *Porphyra columbina* (○, crude protein using conversion factor of 6.25; □, sum of anhydroamino acids; △, biuret). Error bars indicate ±1 standard deviation.

and cysteine as cysteic acid. Three samples were analyzed for taurine and methionine.

Samples of air dried seaweed were oxidized with performic acid to stabilize cysteine as cysteic acid (Hirs 1967). The samples were then hydrolyzed in 6M HCl *in vacuo* for 24 hours at 110°C (Moore *et al.* 1958). The resulting amino acids were measured using a Waters Millipore HPLC Amino Acid Analyzer, with a sulphonated polystyrene column, using a halide buffer system. Post column fluorescence with the addition of hypochlorite was used for the detection of proline. The three samples analyzed for taurine and methionine were not oxidized with performic acid. In this study "total amino acids" refers to the sum of amino acids obtained from acid hydrolysis, and, therefore, consists of protein-bound and free amino acids. The "total amino acid" summations were based on anhydroamino acid molecular weights. Single analyses were carried out. The coefficient of variation (cv^2) was measured for a duplicate sample (*P. columbina*, 9 August 1985). The cv^2 of the sum of the individual amino acids was 8.6%, and this was used as an approximation of the error.

Free amino acids were extracted from the ground, air dried samples (10 mg) in Eppendorf tubes, using 0.5 ml 0.2 M Na citrate/HCl (pH 3.0). Samples of *P. columbina* were sonicated for 30 minutes, whereas *P. subtumens* required 60 minutes. The samples were left standing at ambient temperature for two hours before centrifuging. The amino acids were measured using the Amino Acid Analyzer, as for the acid hydrolysis. Summations were based on the molecular weight of the "free" amino acid form. Single analyses were carried out. The coefficient of variation (cv^2) was measured for a duplicate sample (*P. columbina*, 9 August 1985). The cv^2 of the

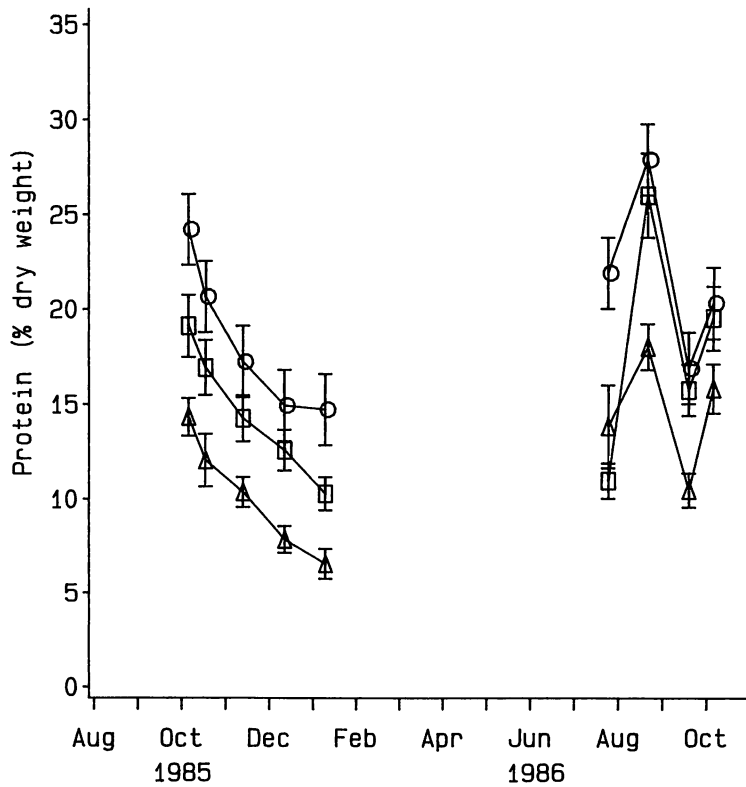

Fig. 1b. Seasonal variation in the protein content of *Porphyra subtumens* (○, crude protein using conversion factor of 6.25; □, sum of anhydroamino acids; △, biuret). Error bars indicate ±1 standard deviation.

sum of the invididual amino acids was 5.0%, and this was used as an approximation of the error.

The water content of the air dried (at 30°C) samples, was determined by drying duplicate portions under vacuum at 110°C until constant weight was achieved ("oven dried"). "Dry weight" in this study refers to "oven dry weight".

Results

Seasonal variation in protein content was observed in *P. columbina* and *P. subtumens* by three methods (Fig. 1a, b). The highest levels occurred in the winter months in both species. Using the same method, the protein contents of the two species were comparable for samples collected at similar times. The protein levels in *P. columbina* samples analyzed ranged from 10.6 to 33.8%, dry weight, when measured by nitrogen multiplied by 6.25; 8.2 to 24.5% by sum of anhydroamino acids (not including Met, Trp, Tau); 6.2 to 21.1% by the biuret method. For *P. subtumens* the ranges were 14.8 to 28.2%, dry weight; 10.2 to 26.0% and 6.5 to 18.0%, respectively. The results of the three methods were correlated (Pearson's correlation, $P<0.05$). However, the average protein content varied significantly between the three methods (Duncan's multiple range test, $P<0.01$). For both seaweeds multiplying nitrogen by 6.25 gave the highest average value (*P. columbina*: 25.5%±7.9%, dry weight and *P. subtumens*: 20.0%±4.5%), followed by sum of anhydroamino acids (17.6%±5.2% and 16.1%±5.0%, respectively (not including Met, Trp and Tau)) and the biuret method (15.3%±4.8% and 12.1%±3.7%, respectively).

Amino acid analysis showed Ala followed by Glu, Asp and Leu were the major amino acids in *P. columbina* (Table 1). In *P. subtu-*

Table 1. Seasonal variation in the amino acid composition of *P. columbina*, St Clair (g/100 g dry weight).

Amino acids	Collection date										
	9 Aug[1]	31 Aug	16 Sep	8 Oct	15 Oct	23 Dec	14 Mar	18 Apr	9 Jun	22 Jul	7 Aug
Ala	3.49	4.82	3.30	2.87	1.65	1.40	1.81	2.08	4.18	4.58	3.50
Arg	1.64	1.47	1.56	1.57	1.16	0.22	0.67	0.98	1.26	1.36	1.27
Asp	2.01	2.54	2.73	1.92	1.68	0.89	1.22	1.38	2.37	2.20	2.38
$CysO_3H$	0.82	0.56	0.77	0.73	0.56	0.29	0.32	0.44	0.50	0.57	0.77
Glu	2.63	2.90	2.77	2.28	1.69	0.76	1.16	1.64	2.73	2.52	2.07
Gly	1.30	1.45	1.31	1.25	1.08	0.70	0.93	0.92	1.40	0.74	0.84
His	0.26	0.36	0.90	0.90	0.77	0.12	0.12	0.13	0.54	0.16	0.73
Ile	0.66	0.70	0.62	0.60	0.49	0.27	0.34	0.51	0.73	0.66	0.65
Leu	1.29	2.63	1.27	0.95	0.51	0.62	0.80	1.03	2.31	2.41	1.32
Lys	1.26	1.22	1.17	1.15	0.97	0.56	0.81	0.88	1.24	1.16	1.25
Met	nd[2]	0.33[3]	nd	nd	nd	nd	nd	nd	nd	0.27[3]	nd
Phe	0.50	0.60	0.48	0.47	0.35	0.19	0.21	0.30	0.50	0.46	0.36
Pro	0.83	0.78	0.88	0.72	0.62	0.28	0.38	0.82	0.78	0.84	0.81
Ser	1.23	1.28	1.23	1.12	0.91	0.49	0.55	0.83	0.97	1.05	1.28
Tau	nd	0.31[3]	nd	nd	nd	nd	nd	nd	nd	0.25[3]	nd
Thr	1.13	1.33	1.15	1.04	0.89	0.53	0.57	0.90	1.04	1.11	1.23
Tyr	0	0.24[3]	0	0	0	0	0	0	0	0.10[3]	0
Val	1.10	1.76	1.10	1.02	0.85	0.85	0.71	0.91	1.05	1.67	1.43
Total	20.13	25.27	21.21	18.59	14.19	8.17	10.59	13.74	21.57	22.11	19.87

[1] Mean of duplicate determinations.
[2] Not determined.
[3] No performic acid treatment.

Table 2. Seasonal variation in the amino acid composition of *P. subtumens*, Brighton (g/100 g dry weight).

Amino acids	Collection date								
	6 Oct	18 Oct	13 Nov	12 Dec	10 Jan	25 Jul	22 Aug	19 Sep	6 Oct
Ala	1.55	1.20	1.08	0.90	0.76	1.00	2.59	1.14	1.35
Arg	1.33	1.51	1.33	1.10	0.76	0.78	1.75	0.74	1.48
Asp	2.62	2.00	1.80	1.97	0.86	1.46	2.81	1.84	2.68
$CysO_3H$	0.64	0.53	0.48	0.51	0.57	0.40	0.71	0.48	0.58
Glu	2.93	2.53	2.01	1.69	1.28	1.57	3.41	2.26	2.75
Gly	1.17	0.89	0.76	0.70	0.67	0.71	1.24	0.82	0.99
His	0.06	0.39	0.23	0.06	0.10	0.02	0.88	0.14	1.06
Ile	0.85	0.73	0.64	0.56	0.54	0.41	1.05	0.68	0.83
Leu	1.52	1.30	1.19	0.99	0.93	0.70	3.00	2.57	1.52
Lys	1.25	1.07	0.98	0.76	0.64	0.93	1.65	0.98	1.23
Met	nd[1]	0.29[2]	nd	nd	nd	nd	nd	nd	nd
Phe	0.54	0.65	0.44	0.22	0.16	0.06	1.61	0.30	0.65
Pro	0.95	0.85	0.64	0.61	0.93	0.64	1.01	0.71	0.86
Ser	1.36	1.06	0.97	0.87	0.63	0.80	1.55	1.00	1.21
Tau	nd	0.32[2]	nd	nd	nd	nd	nd	nd	nd
Thr	1.19	0.91	0.76	0.74	0.62	0.70	1.23	0.82	1.08
Tyr	tr[3]	0.14	tr	tr	tr	tr	0.26	0.22	0.10
Val	1.22	1.12	0.93	0.82	0.80	0.72	1.25	1.00	1.09
Total	19.15	17.48	14.23	12.49	10.24	10.92	26.00	15.69	19.46

[1] Not determined.
[2] No performic acid treatment.
[3] Trace.

mens the same four major amino acids were found (Table 2), but Glu, and not Ala, was the predominant amino acid. Other prominent amino acids found in both seaweeds included Val, Lys and Arg. The levels of amino acids were similar in both species, except for Ala, which was notably higher in *P. columbina*. Tyr was detected only in *P. columbina* when the samples had not been oxidized with performic acid. Therefore, it would appear that Tyr was destroyed in *P. columbina* that had been treated with performic acid prior to the acid hydrolysis. The seasonal fluctuation of most of the individual amino acids was correlated to protein (biuret method), nitrogen and the total amino acids (Pearson's correlation, $P<0.05$).

The total level of free amino acids ranged from 0.5 to 4.1% in *P. columbina* and from 1.0 to 2.3% in *P. subtumens* (not including Met, Trp and Tau) (Tables 3 and 4). For *P. columbina*, the total free amino acids were correlated seasonally to the total amino acids, obtained by acid hydrolysis, and to the nitrogen content (Pearson's correlation, $P<0.05$), but correlation was weak for *P. subtumens*.

Discussion

Seasonal changes in the protein content of *P. columbina* and *P. subtumens* were found (Fig. 1) with the highest values observed in the winter months. Our results are in agreement with the seasonal fluctuation in nitrogen previously reported (Brown *et al.* 1990) for *P. columbina*, in *P. yezoensis* sampled on four occasions (Ji *et al.* 1981) and other *Porphyra* spp. (Takagi 1951). The higher values in winter correspond to the period of maximum growth.

Eighteen amino acids were found in *P. columbina* and *P. subtumens* (Tables 1 and 2) with Ala, Glu, Asp and Leu, the major amino acids. Some loss of Thr and Ser may have occurred because these amino acids can be par-

Table 3. Seasonal variation in the free amino acid composition of *P. columbina*, St Clair (g/100 g dry weight).

Amino acids	Collection date								
	9 Aug[1]	16 Sep	8 Oct	15 Oct*	23 Dec	18 Apr	9 Jun	22 Jul	7 Aug
Ala	1.80	1.51	1.26	0.57	0.17	1.26	2.23	2.22	2.28
Arg	tr[2]	tr	tr	—	tr	tr	tr	tr	tr
Asp	0.30	0.15	0.12	—	0.07	0.19	0.21	0.16	0.16
$CysO_3H$	0.07	0.06	0.05	—	0.02	0.04	0.04	0.05	0.03
Glu	0.68	0.54	0.55	0.30	0.15	0.29	0.68	0.92	0.77
Gly	0.04	0.03	0.04	0.03	0.01	0.06	0.02	0.03	0.03
His	0.02	0.01	0.02	—	0.02	0.02	0.02	0.02	0.02
Ile	0.02	0.02	0.01	—	tr	tr	0.01	0.01	0.01
Leu	0.03	0.03	0.03	—	0.01	tr	0.02	0.03	0.02
Lys	0.04	0.04	0.03	—	0.02	0.03	0.03	0.05	0.03
Met	nd[3]	nd	nd	nd	nd	nd	nd	nd	nd
Phe	0	0	0	—	0	0	0	0	0
Pro	0.31	0.44	0.27	0.21	tr	0.21	0.46	0.42	0.47
Ser	0.17	0.20	0.14	—	0.02	0.10	0.20	0.17	0.11
Tau	nd	nd	nd	nd	nd	nd	nd	0.27	nd
Thr	0.03	0.04	0.03	—	0.02	0.05	0.03	0.03	0.03
Tyr	0	0	0	—	0	0	0	0	0
Val	0.02	0.03	0.03	—	0.01	0.02	0.02	0.02	0.03
Total	3.52	3.08	2.58	—	0.53	2.26	3.97	4.38	3.99

[1] Mean of duplicate determinations.
[2] Trace.
[3] Not determined.
* Insufficient sample.
— denotes inaccurate peak integration.

tially destroyed by acid hydrolysis (Heimann 1980). The amino acid analyses generally agree with what has been reported for other *Porphyra* spp. (Munda and Gubensek 1976; Ji *et al.* 1981; Amano and Noda 1990). The seasonal changes in the individual amino acids present in highest concentrations are similar to those we found for protein by three different methods and are comparable to those observed (Ji *et al.* 1981) for *P. yezoensis*.

The free amino acids Ala, Glu and Gly (Nisizawa *et al.* 1987; Noda *et al.* 1975) and Tau (Noda and Iwata 1978) are thought to contribute to the desirable flavour of nori. *P. columbina* attained higher levels of Glu, Ala and total free amino acids than *P. subtumens* (Tables 3 and 4). Tau was present in reasonable quantities in both species (0.21 g/100 g, dry weight, in *P. subtumens* and 0.27 g/100 g in *P. columbina*). Harada (1988) recorded 0.48 g/100 g, dry weight, for Tau in *P. columbina*. Noda *et al.* (1975) reported levels of 1.21 to 1.62 g/100 g, dry weight, in *P. tenera*. Harada *et al.* (1990) found Tau varied seasonally (December to April, Japan) in *Porphyra* spp. from approximately 0.80 to 1.50 g/100 g, dry weight. Gly was not a prominent free amino acid in either species in the present study.

It is not surprising that the three protein methods we used gave different results, as they each measure a somewhat different group of compounds. The higher crude protein values could be due to the fact that non-protein nitrogen is present and the conversion factor of 6.25 is not appropriate. The biuret method generally gave the lowest values. In theory the biuret method would under-estimate the protein content, because it does not include free amino acids or dipeptides and relies on protein solubility. The most accurate estimation of protein would be obtained with

Table 4. Seasonal variation in the free amino acid composition of *P. subtumens*, Brighton (g/100 g dry weight).

Amino acids	Collection date							
	18 Oct*	13 Nov	12 Dec	10 Jan*	25 Jul	22 Aug	19 Sep	6 Oct
Ala	—	0.14	0.11	0.04	0.59	0.18	0.13	0.23
Arg	—	tr[2]	tr	tr	tr	tr	tr	tr
Asp	—	0.06	0.09	0.03	0.29	0.07	0.16	0.18
$CysO_3H$	—	0.13	0.06	—	0.09	0.12	0.02	0.03
Glu	0.51	0.41	0.40	0.09	0.78	0.60	0.56	0.55
Gly	0.02	0.01	0.01	0.01	0.04	0.02	0.01	0.01
His	—	0.04	0.02	—	0.01	0.01	0.02	0.02
Ile	—	0.02	0.02	0.01	0.03	0.04	0.02	0.02
Leu	—	0.03	0.04	0.02	0.03	0.07	0.04	0.04
Lys	—	0.04	0.04	0.03	0.05	0.07	0.06	0.06
Met	nd[1]	nd	nd	nd	nd	nd	nd	nd
Phe	—	tr	tr	tr	tr	tr	tr	tr
Pro	—	tr	tr	tr	tr	0.10	tr	tr
Ser	0.04	0.06	0.06	0.02	0.14	0.14	0.08	0.12
Tau	0.21	nd	nd	nd	nd	nd	nd	nd
Thr	—	0.03	0.02	tr	0.07	0.03	0.02	0.02
Tyr	0.08	0.08	0.05	tr	0.14	0.21	0.26	0.11
Val	0.03	0.03	0.09	0.01	0.03	0.06	0.09	0.08
Total	—	1.09	1.01	—	2.30	1.71	1.47	1.48

[1] Not determined.
[2] Trace.
* Insufficient sample.
— denotes inaccurate peak integration.

the knowledge of the molecular weights of the "in-chain" sequence of the amino acids. However, this is impracticable and the sum of anhydroamino acids was chosen for this study. Thus the total level of amino acids will be under-estimated. Free amino acids, that were inclusive in the hydrolysis, were summed as anhydroamino acids. This represents another source of under-estimation of total amino acids. Other investigators generally did not state whether amino acids were summed in anhydroamino or free form. Exceptions are Mukai *et al.* (1981) who used anhydroamino acids and Rosell and Srivastava (1985) who used the free form. In the present study it appeared that the sum of amino acids gave the most accurate protein measurement. However, the method did not include Trp. Literature values for Trp range from 0.11 g/100 g, dry weight, in *P. columbina* (Quilhot 1970) to 0.78 g/100 g in *P. tenera* (Arasaki and Mino 1973). Protein quality based on the amino acid composition, "amino acid score" or "chemical score", (using FAO/WHO/UNU (1985) suggested requirements for adults) was adequate at maximal winter levels in *P. columbina*. When egg was used as a reference protein, as recommended by Passmore and Eastwood (1986), Phe and Tyr were limiting throughout the year in both *Porphyra* species. The egg composition of Paul and Southgate (1978) was used. The amino acid score ranged from 12 to 19, air dried weight, in *P. columbina* and from 3 to 65 in *P. subtumens*. Air dried weight was used in order to approximate nori, which has a similar water content (9 to 12%). The consumption of nori in Japan has been reported to average 72 sheets per capita, per year (Freeman 1985). Consumption would be higher for some individuals than others, but because each sheet weighs only about 3 grams (Miura

1975), nori would not appear to be a major source of protein.

The conversion factors for nitrogen to protein obtained by summation of amino acids, ranged from 3.08 to 5.96 for *P. columbina* and *P. subtumens* (not including Tau, Met and Trp). When the measurements for Tau and Met were included (Tables 1 and 2), as well as an average literature data of 0.43 ± 0.34 g/100 g, dry weight, for Trp (data from Quilhot 1970; Rao and Polacchi 1972; Arasaki and Mino 1973), a conversion factor of 5.0 was obtained. This estimated conversion factor was lower than the factor 6.25, frequently used by researchers studying seaweed, but compares favourably with conversion factors that we have calculated from literature data, using nitrogen in *Porphyra* spp. and corresponding sum of amino acids. These ranged from 3.47 for *P. tenera* (Arasaki and Mino 1973) to 6.17 (Mukai *et al.* 1981) for *P. tenera*, with an average of 4.98. These conversion factors do not contain Tau, and often do not contain Met or Trp.

Rosell and Srivastava (1985) found that multiplying the nitrogen content of the seaweed residue by 6.25, after alcohol extraction (80% ethanol), gave results comparable to those obtained by summation of amino acids. Ito and Hori (1989) noted that 70% ethanol extraction removes soluble nitrogen compounds, including free amino acids, amines and pigments. These usually account for 10 to 20% of the total nitrogen compounds. Leung *et al.* (1972) reported that several seaweeds contained approximately 20% non-protein nitrogen. This indicates 80% of the nitrogen in seaweed is protein nitrogen. Therefore, once again, a factor of 5.0 multiplied by total nitrogen would appear to give a satisfactory estimate of protein content. We conclude, the best method for determining protein levels would be the summation of amino acids, followed by using the conversion factor of 5.0 for the nitrogen value.

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K. A. Aitken・L. D. Melton・M. T. Brown*：ニュージーランド海藻 *Porphyra columbina* Mont. と *Porphyra subtumens* J. Ag.（紅藻植物）におけるタンパク質量の季節変化

紅藻の *Porphyra columbina* Mont. と *Porphyra subtumens* J. Ag. の組織中のタンパク質量の季節変化を測定した。分析方法としては，全窒素量の6.25倍法，Biuret 法，アンヒドロアミノ酸量の合計法の 3 種の方法を用いた。両種 *Porphyra* のタンパク質量は似たような季節変化を示し，冬期に最大となった。タンパク質量は測定法により異り，常法に従って全窒素量を6.25倍して得た値が最も高い値となり，次いで総アンヒドロアミノ酸法による値がつずき，Biuret 法では最小の値となった。このうち，二番目の方法が *Porphyra* タンパク質の定量法としては最も正確なものと思われた。この方法によって得られた結果に基づくと，全窒素量を5.0倍することにより，より正確なタンパク質量が求められることが提唱できる。2 種の *Porphyra* の主要アミノ酸は Ala, Glu, Asp, Leu で，次いで Val, Lys, Arg であった。そのうち Ala は特に *P. columbina* の主要なものであるのに対して，*P. subtumens* では Glu が主であった。これと同じことが両種 *Porphyra* の遊離アミノ酸においてもみられた。またタンパク質構成アミノ酸と主な遊離アミノ酸の季節変化は，それぞれ特異的な様相を示した。(Food Science Department, University of Otago, P.O. Box 56, Ounedin, New Zealand *Botany Department, University of Otago, P.O. Box 56, Dunedin, New Zealand)