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Ascorbic acid in the New Zealand seaweeds *Porphyra columbina* Mont. and *Porphyra subtumens* J. Ag. (Rhodophyceae)

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Ascorbic acid in *Porphyra columbina* and *P. subtumens* (Rhodophyceae) was determined by two independent techniques: high performance liquid chromatography (HPLC) and titration with 2,6-dichlorophenolindophenol. Variations in the levels of ascorbic acid in the two species were investigated during the period of active growth. Values obtained on freshly harvested seaweed ranged from 402 to 186 mg 100 g^{-1} dry weight for *P. columbina* and from 274 to 187 mg 100 g^{-1} dry weight for *P. subtumens*. After seven months storage the ascorbic acid content of the dried seaweeds had decreased to less than 100 mg 100 g^{-1} dry weight in *P. columbina* and to less than 30 mg 100 g^{-1} dry weight in *P. subtumens*. Nori sheets were made from *P. columbina* and toasted without detectable loss of ascorbic acid.

*Key Index Words: ascorbic acid—2,6-dichlorophenolindophenol—New Zealand—HPLC—nori—*Porphyra columbina—Porphyra subtumens—*Rhodophyceae.*

Porphyra, a cosmopolitan genus, is eaten in many parts of the world but is particularly important in Japan and China where it is popularly known as nori and zicai, respectively. In these countries seven species are grown commercially with *P. yezoensis* Ueda, *P. tenera* Kjellman and *P. haitanensis* T.J. Chang *et* Zheng Baofu being the most important (Miura 1975, Tseng 1981, Nisizawa *et al.* 1987, Tseng and Fei 1987).

In New Zealand the two most widely distributed species of *Porphyra* are *P. columbina* Mont., a winter annual, and *P. subtumens* J. Ag., reported to be a summer annual (Chapman 1969). However, our observations on *P. subtumens* indicate it is found throughout the year with a plentiful number of larger plants in September and October while there were only small plants present between February and April (Melton and Brown unpublished observations). *P. columbina* grows in the intertidal zone of rocky shores along most of the New Zealand coastline whereas *P. subtumens* is an epiphyte growing on *Durvillaea antarctica* (Chamisso) Hariot and *D. willana* Lindauer in the upper sublittoral. *P. columbina*, known as karengo, is eaten by the Maori and is being considered for aquaculture. Recent investigations on *P. columbina* have been concerned with the structure of the porphyran (Brasch *et al.* 1981a, 1981b, 1984), cell culture (Liu and Gordon 1987) and resistance to desiccation (Brown 1987).

Analyses of several species of *Porphyra* indicate that they contain significant amounts of proteins, minerals and vitamins essential for human nutrition (Levring *et al.* 1969). Vague claims as to the vitamin C content of nori sheets such as that it contains more than the juice of tangerines and lemons (Noda and Iwata 1978) and one and a half times the content of oranges (Chapman and Chapman 1980) have no accompanying analytical evidence to support them. The analytical evidence which does exist shows a large varia-

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tion in values: 10–831 mg 100 g^{-1} for dried *P. tenera* (KANAZAWA 1963), 112.5 mg 100 g^{-1} for nori sheets (hoshi-nori) (NISIZAWA *et al.* 1987). Differences in ascorbic acid levels between genera of the Rhodophyceae have been reported (LUNDE and LIE 1938; CREAC'H 1960; MUNDA 1987). Seasonal variation has been observed within species (LUNDE and LIE 1938; MUNDA 1987) including *Porphyra umbilicalis* (L.) J. AG. (LUNDE and LIE 1938). Furthermore vitamin C values decreased in dried *P. yezoensis* (ARAKI *et al.* 1982; OGAWA *et al.* 1983) and in nori sheets (OOHUSA 1984) during storage.

The aim of the present study was to determine the ascorbic acid contents of two New Zealand *Porphyra* species. Levels in dried seaweeds immediately after collection and on storage were determined. The effect of making nori sheets on the ascorbic acid contents was also investigated. Two independent analytical procedures, HPLC and 2,6-dichlorophenolindophenol titration, were used.

Material and Methods

Samples of *Porphyra columbina* were collected at intervals from June to October 1986 (winter, spring) from Brighton (45°57'S, 170°20'E) and on three occasions from St Clair (45°55'S, 170°29'E) near Dunedin, New Zealand. The two locations are exposed, coastal sites 14 km apart. The epiphyte, *Porphyra subtumens* was collected only from the Brighton site over the same period of time. Plants were picked off *Durvillaea antarctica* and *D. willana* while uncovered at low tide. Whole plants were collected for both seaweeds and were washed with seawater at the point of collection to remove sand and other debris.

Freshly collected samples were dried at 30°C to a water content of approximately 8% in a circulating air oven. Drying commenced within 3 hr of collection. Dried samples were stored under air in sealed plastic bags in the dark at *ca.* 18 to 20°C. Samples (5 to 10 g) equivalent to 20 to 40 *P. columbina* plants or at least 120 *P. subtumens* plants were ground on the day of analysis in a Falling Number mill (Type 3303, Huddinge, Sweden, zero setting) to a fine powder.

Ascorbic acid (AA) was extracted from a portion of the ground seaweed (0.3 to 2.0 g) by shaking for three minutes with the appropriate extracting solution (100 m*l*). Preliminary work showed that increasing the extraction time to 6 minutes or doing 2 successive extractions did not increase the yield of AA. The suspension was filtered through glass wool and then through filter paper (Whatman No. 4).

Ascorbic acid analyses were performed on 7 m*l* aliquots using the titrimetric method (AOAC 1984) with metaphosphoric-acetic acid as the extracting solution and the 2,6-dichlorophenolindophenol reagent diluted 1 in 5. Analyses were also performed following the HPLC method of WIMALASIRI and WILLS (1983) with 3% citric acid as the extracting solution. The HPLC system comprised a Waters Associates 6000A pump, a U6K injector, a Lambda-Max Model 480 spectrophotometer (Waters Associates) set at 254 nm, and a Shimadzu integrator recorder. The column was a Waters NH_2 μ Bondapak. The flow rate was 2 m*l* min^{-1}. The mobile phase was 70 : 30 acetonitrile to water with 10.0 mM potassium dihydrogen phosphate with the pH adjusted to 4.3 using concentrated phosphoric acid. A standard curve was constructed using five AA concentrations of 5 to 25 μl ml^{-1}. Samples were passed through a C_{18} Sep-pak (Waters Associates), which had been previously washed first with 4 m*l* methanol and then with 10 m*l* distilled deionised water, before injection into the HPLC. Each sample (50 μl) was injected four times.

Repeatability was assessed by repeating the extraction and analysis of samples of *Porphyra* from the same collection eight times on the same day.

Recovery tests were performed by addition of AA (2.5 μg ml^{-1} and 5.0 μg ml^{-1}) to the sample in the extracting solution and proceeding as before. The AA present was compared with an identical sample which did not have AA added to the extracting solution.

Ascorbic acid determinations were carried out on the sample immediately after drying at 30°C and at intervals varying from one to several weeks. Water determinations were also performed at the same time. These were graphed against number of days of storage, and the line of best fit drawn from the linear regression. Water values used for any particular day were read off this graph.

Nori sheets "hoshi-nori" were made from samples of *P. columbina* collected from Warrington beach, north of Dunedin (45°43'S, 170°36'E), on 22 February 1987. The seaweed was kept in seawater at 4°C until it was processed. Nori sheets were prepared by blending wet seaweed with HPLC grade water at 5°C in a blender (Waring, New Hartford, USA) for 30 seconds until small pieces (5 mm across) were obtained. The suspension was immediately poured onto a wire mesh mat covered with muslin gauze (supported by a wooden frame, 24 cm × 24 cm) and spread out to form a sheet approximately 5 mm thick. Excess water was removed by pushing the mat down with the fingertips. The tray was placed in an oven with circulating air at 30°C for three to five hours until dry. The sheet of nori was toasted by moving it back and forth approximately 8 cm above a Bunsen flame. It took approximately 15 seconds for the colour to change to bright green which indicates the toasting is complete (CHAPMAN and CHAPMAN 1980). The HPLC and water analyses were done on the fresh wet *P. columbina* (weighing the sample immediately after spinning three revolutions in a plastic salad drier), on the fresh dried sample (30°C, four hours), on the blender water, on the final nori product and on the nori after toasting.

All AA values given in this paper are expressed as mg AA per 100 g dry weight. The dry weight was obtained by drying the samples for approximately 16 hours in vacuum oven at 115°C until constant weight was achieved. This is not to be confused with the initial drying process for 6 to 36 hours at 30°C, after which the seaweed had a moisture content of approximately 8%. No AA values have been expressed in these latter terms.

Results and Discussion

During July and August, *Porphyra columbina* from Brighton had higher AA levels than *P. columbina* from St Clair (Table 1). The value obtained for St Clair remained relatively constant (~200 mg AA 100 g^{-1} dry weight) whereas at Brighton a peak value was observed in August (402 mg AA 100 g^{-1}) and declined thereafter. Differences between the two sites may be related to different environmental conditions although light, temperature and salinity were similar. The growth period did, however, appear to differ. The St Clair plants were evidently earlier, were at a more advanced stage of growth and were more plentiful at any particular time up to and including July. The maximum AA values in plants from Brighton corresponded with the time the seaweed was tender, relatively large, and prior to yellowing. This would appear to be the optimal time for collecting *Porphyra* from this site to produce nori sheets. The

Table 1. Changes in ascorbic acid in *Porphyra columbina* collected from the midlittoral of Brighton and St Clair and in *Porphyra subtumens* from the sublittoral fringe of Brighton. All values are in mg AA 100 g^{-1} dry weight. Standard deviations are given in brackets.

Sample date and site	Ascorbic acid	
	HPLC	Titration
P. columbina Brighton		
25 July 1986	322 (15)	342 (3)
22 August 1986	376 (13)	402 (3)
19 September 1986	304 (10)	293 (5)
6 October 1986	253 (2)	249 (4)
P. columbina St Clair		
9 June 1986	199 (6)	205 (1)
22 July 1986	186 (18)	207 (2)
7 August 1986	195 (10)	180 (2)
P. subtumens Brighton		
14 July 1986	220 (13)	233 (2)
22 August 1986	187 (13)	196 (4)
19 September 1986	261 (14)	274 (5)
6 October 1986	263 (11)	251 (2)

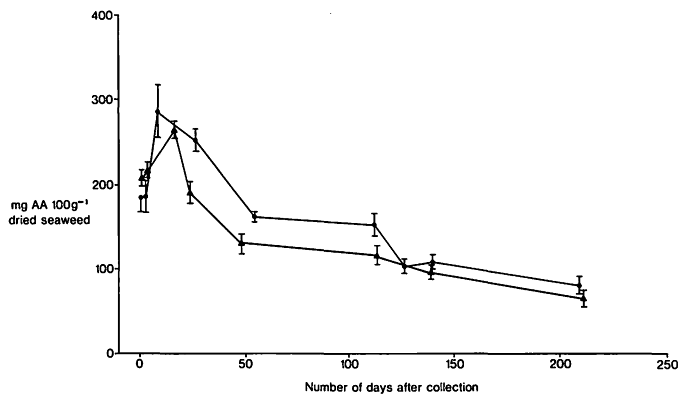

Fig. 1. Effect of storage on ascorbic acid (AA) content of *Porphyra columbina* collected from St Clair on 22 July 1986. HPLC data ●. Titration with 2,6-dichlorophenolindophenol data ▲. Error bars indicate ±1 standard deviation.

high AA values at this stage correlate with LISO and CALBRESE's (1974) finding that AA levels in red algae were significantly higher in actively growing parts of the plant.

Porphyra subtumens from Brighton had noticeably lower AA levels than *P. columbina* from the same site in the months of July and August (Table 1). In contrast in September and October values rose to be comparable to *P. columbina* values. While CHAPMAN (1969) considered *P. subtumens* to be a summer annual, our own observations indicate rapid growth during September and October, the time of higher AA values.

Porphyra columbina collected from both St Clair and Brighton sites, in July, showed a marked decrease in AA levels during storage of the dried samples (Figs. 1 and 2). Similarly, AA levels in *P. subtumens* decreased on storage (Fig. 3). Indeed, all dried samples of *P. columbina* and *P. subtumens*, no matter when collected, showed decreases in AA on storage (FRIEDLANDER 1987). The rate of destruction of AA is influenced *inter alia* by the temperature, the presence of oxygen and the water activity of food (TANNENBAUM *et al.* 1985). Considerable losses of AA in Japanese dried *P. yezoensis* and nori sheets stored in air have previously been reported (ARAKI *et al.* 1982; OGAWA *et al.* 1983;

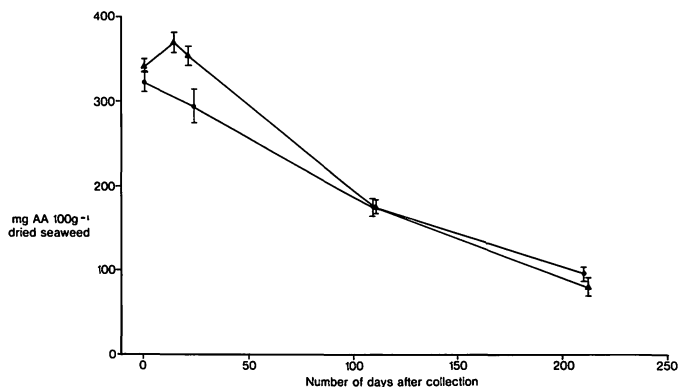

Fig. 2. Effect of storage on ascorbic acid (AA) content of *Porphyra columbina* collected from Brighton on 25 July 1986. HPLC data ●. Titration with 2,6-dichlorophenolindophenol data ▲. Error bars indicate ±1 standard deviation.

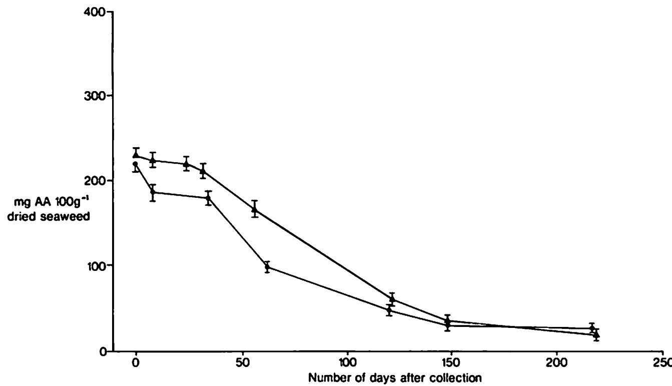

Fig. 3. Effect of storage on ascorbic acid (AA) content of *Porphyra subtumens* collected from Brighton on 14 July 1986. HPLC data ●. Titration with 2,6-dichlorophenolindophenol data ▲. Error bars indicate ±1 standard deviation.

OOHUSA 1984). The 3 groups of investigators found loss of AA was directly related to the water activity. In our investigation, after the initial drying process at 30°C the seaweeds reached a water content of 6–10%. On storage the water content increased steadily to approximately 10–13% after 150 days. Ascorbic acid calculations were adjusted accordingly, however this increase in water content undoubtedly contributed to the decrease in AA values observed during the storage period.

The HPLC and titrimetric techniques produced comparable results (Table 1, Figs. 1, 2 and 3). The coefficient of variation for both methods was 3–5%, as determined by repeatability trials. Recovery tests gave a 107% recovery for HPLC and 81% for titration. JENSEN (1963) showed the titrimetric method for determining AA in brown seaweeds produced unrealistically high values unless phenolic compounds in the seaweed, which interfere with the titration, are removed prior to analysis. In the present study the effect of phenolics was not investigated. Although the recovery was only 81% by the titration method, it is possible that phenolic compounds increased the AA values, thus fortuitously giving values comparable to those obtained by HPLC.

The AA levels obtained in the present study fall within the range of 10–831 mg 100 g^{-1} previously determined for *Porphyra tenera* (KANAZAWA 1963) and are higher than the value of 112.5 mg 100 g^{-1} reported for nori sheets (hoshi-nori) made probably from *Porphyra yezoensis* (NISIZAWA *et al.* 1987). Comparisons with values for *P. umbilicalis* (LUNDE and LIE 1938) which are quoted per wet weight without giving a water content, are not possible since the water content of fresh seaweed varies markedly depending on whether the seaweed is shaken to remove excess water or blotted dry.

No significant change in AA levels was noted on drying of the wet seaweed (30°C, 4 hours). Nor did nori sheet production or subsequent toasting significantly alter AA values (Table 2). Loss of AA might have been expected during nori sheet production since the *Porphyra* was blended with water, and AA is water soluble. However, analysis of the water initially drained from the nori sheets showed that leaching of AA was not detected. The volume of water used is important with respect to AA losses (EHEART and GOTT 1965). The present study used 100 to 150 m*l* of water per 10 g wet *Porphyra*. The authors were unable to find a reference to the seaweed to water ratio used in nori making so a comparison could not be made.

Ascorbic acid is generally considered to be heat sensitive. The nori sheets were toasted at what was assumed to be high temperature

Table 2. Ascorbic acid levels during nori sheet making using the same *Porphyra columbina* sample on two different occasions. All values in mg AA 100 g^{-1} dry weight.

	25 February 1987	2 March 1987
Wet seaweed	298*a*	251*b*
Freshly dried seaweed	311*a*	—
Nori sheet	325*a*	248*b*
Water drained from nori sheet	ND	ND
Nori sheet toasted	329*a*	233*b*

Values are the mean of four HPLC injections. ND=Not detected. Different letters (*a, b*) between treatments represent significant differences ($P<0.01$).

for only 15 seconds. Hence, it is noteworthy that no loss of AA was observed on toasting the nori sheets (Table 2).

Therefore, nori sheets can contain as much AA as the seaweed itself providing the seaweed used is fresh, chopped in minimal water, dried and toasted for a minimal length of time and consumed relatively soon after making.

HPLC analysis on Japanese nori ("Westbrae Natural" brand sushi-nori) purchased in Dunedin could detect no AA. This was not surprising since the sample would have been over twelve months old before analysis, due to importing and storage. The absence of AA is not unexpected as some values reported by MIURA (1975) for Japanese nori sheets are very low (2 mg 100 g^{-1}).

It must be borne in mind that a sheet of nori weighs *ca.* 3.0 g. Therefore the AA present in one sheet of nori, even if the nori is freshly prepared or stored in the absence of oxygen, will only make a small contribution to an individual's AA nutritional requirements. However, if *P. columbina* was eaten in larger quantities, either freshly harvested, or in a recently dried form, or as freshly made nori sheets, it would make an important contribution to human AA requirements.

Conclusions

Claims of high levels of AA in dried *Porphyra* have been confirmed for *P. columbina* and *P. subtumens* but the actual values are dependent upon species, location, time of collection and length of storage. The maximum levels of AA in *P. columbina* occur during the phase of vigorous growth when the seaweed is tender and richly pigmented. This would appear to be the optimal time for harvesting. Differences between collection dates were also found in *P. subtumens.* During the period of sampling *P. columbina* from the same site generally had higher or similar AA values to *P. subtumens.*

The method of storage has an important influence on conserving the ascorbic acid content of dried *Porphyra.* When stored under air in plastic bags at *ca.* 18–20°C for 7 months all dried samples of *P. columbina* and *P. subtumens* showed marked decreases in AA levels.

Our method of processing *P. columbina* into nori sheets and its subsequent toasting did not affect the AA content. However, further investigations are required to find ways of maintaining the high levels of AA in the nori sheets during storage.

The HPLC and 2,6-dichlorophenolindophenol titrimetric methods have been commonly used to determine AA contents of foods. However, we believe this is the first time they have both been used to measure AA levels in the same seaweed samples. Comparable data were obtained by the two analytical procedures, but this may have been fortuitous.

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S. F. FRIEDLANDER* · L. D. MELTON* · M. T. BROWN**：ニュージーランド産紅藻 *Porphyra columbina* MONT. 及び *Porphyra subtumens* J. AG. のアスコルビン酸

紅藻 *Porphyra columbina* と *P. subtumens* のアスコルビン酸を高速液体クロマトグラフィー及び2,6-ジクロロフェノールインドフェノールによる適定で定量し，両種の成育の活発な時期におけるアスコルビン酸レベルの変動を調べた。採集直後の試料のアスコルビン酸含量は，*P. columbina* では 402–186 mg 100 g^{-1}（乾重），*P. subtumens* では 247–187 mg 100 g^{-1}（乾重）であた。7カ月貯蔵した試料では，アスコルビン酸含量の減少が認められ，*P. columbina* では 100 mg 100 g^{-1}（乾重）以下に，*P. subtumens* では 30 mg 100 g^{-1}（乾重）以下になっていた。*P. columbina* をすいて焼海苔を作ったが，検出できるほどのアスコルビン酸の減少はなかった。(*Food Science Department, University of Otago, P.O. Box 56, Dunedin, New Zealand; **Botany Department, University of Otago, P.O. Box 56, Dunedin, New Zealand)