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Nutritional Evaluation of Tropical Green Seaweeds *Caulerpa lentillifera* and *Ulva reticulata*

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ABSTRACT

Studies were conducted to evaluate nutritional qualities of two edible green seaweeds, *Caulerpa lentillifera* and *Ulva reticulata*, with a view to their utilization in human nutrition. The proximate composition, mineral and vitamin contents, free fatty acid, and amino acid profiles were investigated. Protein and ash contents were the two most abundant components in these seaweeds. *Caulerpa lentillifera* and *Ulva reticulata* contained 12.49%, 21.06% protein and 24.21%, 17.58% ash based on dry weight, respectively. Both seaweeds contained high amounts of minerals and balanced amino acid profiles. Regarding the Dietary Reference Intake, both kinds of seaweeds were notably rich in iodine. *Caulerpa lentillifera* was also rich in phosphorus, calcium, magnesium and copper, while *Ulva reticulata* was rich in potassium, manganese and ferrous. Comparisons to corresponding nutrient values in other seaweeds and some commonly consumed local vegetables, both seaweeds showed their potential of being health food for human diets or as source of ingredients with high nutritional values.

Key words: *Caulerpa lentillifera*, *Ulva reticulata*, seaweed, nutritional value, protein

INTRODUCTION

Seaweeds are major coastal resources which are valuable to human consumption and environment in many countries. Edible seaweeds were widely consumed, especially in Asian countries as fresh, dried, or ingredients in prepared foods. Compared to land plants, the chemical composition of seaweeds has been poorly investigated and most of the available information only deals with traditional Japanese seaweeds (Fujiwara-Arasaki *et al.*, 1984; Nisizawa *et al.*, 1987). The chemical composition of seaweeds varies with species, habitat, maturity and environmental conditions (Ito and Hori, 1989). In general, seaweeds are rich in non-starch polysaccharides, minerals and vitamins (Darcy-Vrillon, 1993; Mabeau and Fleurence, 1993). As seaweed polysaccharides cannot be entirely digested by human, they are regarded as a new source of dietary fiber and food ingredient. Together with their low lipid content, seaweeds only provide a very low amount of energy. Consumption of seaweeds can increase the intake of dietary fiber and lower the occurence of some chronic diseases (Southgate, 1990).

Although the seaweed floras in Thailand are extensively found, they are relatively under-utilized. Most of them are mainly used as animal feeds and fertilizers by the coastal villagers. The genus *Caulerpa* is common seaweed in tropical and subtropical water. Within this genus, *Caulerpa*

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lentillifera is one of the favored species due to its grass-green in color, soft, and succulent texture and usually consumed in the form of fresh vegetable or salad. It can be cultivated in ponds and open lagoon in the Philippines. In Thailand, many shrimp farms cultivate *Caulerpa lentillifera* in the pond for the purpose of water treatment. *Ulva reticulata* is also one type of green seaweed which is under-utilized. It is widely spread in the southern part of Thailand, especially in Pattani bay and the coastal villagers use it as animal feeds. Both types of green seaweeds are rarely promoted for the increase use for food. One major limitation is the lack of nutritive value of these types of algae. To our knowledge, the nutritional data of both green algae is not yet available. Thus, the aims of this work were to determine the nutritionl compositions of *Caulerpa lentillifera* and *Ulva reticulata*.

This paper presented data on the nutritional and chemical composition of *Caulerpa lentillifera* and *Ulva reticulata;* i.e. proximate composition, mineral, vitamin, fatty acid, and amino acid contents. This work also reported a comparative evaluation of nutritive values of these seaweeds with those of some other seaweeds and some locally consumed vegetables. The potential of both seaweeds as sources of food nutrients was discussed.

MATERIALS AND METHOD

2.1 Collection and preparation of samples

The *Caulerpa lentillifera* seaweed was collected from culture ponds of coastal aquaculture station in Amphor BanLam, Petchburi province, in March. The *Ulva reticulata* seaweed was collected from coastal area of Pattani Bay, Pattani province, in May. The samples taken were washed in running water and divided into three portions. For vitamin analysis, fresh sample were taken immediately by covering with black sheet and the analysing steps were carried out with minimum delay. For fatty acid composition, the freeze dried samples were used. For the rest of the analyses, dry samples were used. The dry samples were prepared by drying the fresh seaweeds in the hot-air oven at 60 ∂C until dry and kept in air-tight plastic bag in desiccators at room temperature (25 ∂C) for further analysis.

2.2 Analytical methods

2.2.1 Proximate analysis

Total nitrogen, fiber, and ash contents were determined by standard AOAC (1990) methods. Fat content was determined according to Bligh and Dyer method (Bligh and Dyer, 1959). Total protein content was calculated by multiplying Kjeldahl nitrogen by 6.25. Ash content was conducted by ashing the ground dried samples overnight in muffle furnace at 525 ∂C. Crude fiber analysis was determined by filtering with a Fiber-Tec system.

2.2.2 Mineral contents

For the determination of mineral elements (phosphorus, potassium, calcium, magnesium, zinc, manganese, ferrous, copper and iodine), triplicate determinations of each element were carried out. The methods of analysis were as followed:

P by Vanadomolybdophosphoric yellow method

K Ca Mg by Atomic Absorption with wet digestion (H_2SO_4-Se)

Zn Mn Fe Cu by Atomic Absorption with wet digestion (H_2ClO_4-HNO_3 3:5)

I by Spectrophotometric Kinetic Assay Method

2.2.3 Vitamin contents

Vitamin determinations were conducted by the Nutrition Division, Department of Health, Ministry of Public Health. Methods of Analysis were as followed (Nutrition Division, 2001):

Vitamin A/Carotene by HPLC method
Thiamin by Thiochrome method
Riboflavin by Spectrofluorometric method

Niacin by Microbiology method
Ascorbic acid (Total Vit C) by 2,4 dinitro-phenylhydrazine method
Vitamin E (Alpha-tocopherol) by HPLC method

2.2.4 Fatty acid compositions

Fatty acids were determined by gas chromatographic quantification of their metyl esters (FAMES), which were prepared by extraction and transmethylation (modified Bligh and Dyer method, 1959). FAMES samples were analysed using capillary gas chromatography (GC-17A, Shimadzu/ Japan) equipped with a DB-WAX;J&W capillary column (30m ¥ 0.25mm, film thickness 0.25 µm) and an FID Detector. The injection and detection temperature were 250∂C and 270 ∂C with split ratio of 1:100 using helium as carrier gas. The running method was through a temperature gradient from 150 ∂C up to 250 ∂C with an increase rate of 8.0 ∂C/min. Identification of fatty acids in the samples were performed by comparing their retention times with those of a standard mixture (C_{14}-C_{24} fatty acids) and peak areas were calculated from the total identified fatty acids area and the average values of two injections of each duplicate extracts.

2.2.5 Amino acid compositions

Amino acid analysis was perfomed using the Waters Associates AccQ-TAG method (Liu *et al*.,1995). This technique comprises of three steps (i) hydrolysis by 6NHCl at 110∂C for 22 hours (ii) pre-column derivatization of samples with AccQ-fluor reagent and (iii) analysis by (reverse phase) HPLC. The chromatographic separation was performed using WATERS Alliance 2695 with heater, WATERS 2475 Multi l fluorescence detector (EX:250, EM:395 nm) and ACCQ-TAG column (3.9¥150 mm, particle size 4 µm). The solvent system consisted of two eluents: (A) AccQ-TAG Eluent A and (B) Acetonitrile in water. A set of amino acid standards (Sigma chemicals) was analysed with each set of experimental samples. The experiment was performed in duplicate. Identification of the amino acid in the sample was carried out by comparison with retention times of the standards.

RESULT AND DISCUSSION

Proximate analysis

The proximate composition based on dry weight of *Caulerpa lentillifera* and *Ulva reticulata* were shown in Table 1. It was found that the protein content of both seaweeds differed according to species. The protein contents of both samples (12.49-21.06%) were within the range of 10-47% for green seaweeds reported by Fleurence (1999). The protein content in *Ulva reticulata* was almost twice of that found in *Caulerpa lentillifera* and was notably higher than that of *Ulva lactuca*. Variation in protein content of seaweeds can be due to different species, seasonal period and geographic area.

Compare to those reported in other seaweeds, the protein content of *Caulerpa lentillifera* (12.49%) was comparable to the red algae *Palmaria sp*. (13.87%), notably higher than

Table 1 Proximate composition (g/100g sample dry basis) of *Caulerpa lentillifera* and *Ulva reticulata*.

Composition	*Caulerpa lentillifera*	*Ulva reticulata*
Crude protein (N factor = 6.25)	12.49±0.3	21.06±0.42
Crude lipid	0.86±0.10	0.75±0.05
Crude fiber	3.17±0.21	4.84±0.33
Ash	24.21±1.7	17.58±2.0
Carbohydrate[a]	59.27	55.77
Moisture	25.31±1.15	22.51±0.97

[a] Calculated by difference (= 100–crude protein-crude lipid -ash-crude fiber)

some green algae *Ulva lactuca* (7.06%) (Wong and Cheung, 2000), some brown algae (e.g. *Himanthalia elongata* (7.49%) and *Laminaria ochroleuca* (7.49%)), but was about half of that reported in *Porphyra sp.* (24.11%) (Sanchez-Machado *et al*., 2004). For *Ulva reticulata*, the protein was three times higher than that contained in the same genus *Ulva lactuca* but slightly lower than that of *Porphyra sp.* (Sanchez-Machado *et al*., 2004). However, it should be noted that the protein content of seaweeds varied not only between species but also between seasons (Fleurence, 1999).

Ash contents of both seaweeds found in the level of 17-24% were considerably high. High level of ash was associated with the amount of mineral elements. Ash content in *Caulerpa lentillifera* (24.21%) was higher than that found in *Ulva reticulata* (17.58%). The amount found were comparable to those reported in other species i.e., *Himanthalia elongata* (26.78%), *Laminaria ochroleuca* (29.47%) and *Porphyra sp.* (19.07%) (Sanchez-Machado *et al*., 2004). Generally, the ash content of seaweeds are much higher than those of terrestrial vegetables other than spinach (Sanchez-Machado *et al*., 2004).

The total lipid contents in both samples (0.75-0.86%) were found relatively low which were in accordance with 0.7-1.05% as reported in red and brown algae mentioned above (Sanchez-Machado *et al*., 2004). Typically, seaweeds are not considered to be good source of lipid.

Mineral contents

The mineral contents of both seaweeds as well as the values reported in local vegetables and selected edible seaweeds were shown in Table 2. The Dietary Recommended Intake (DRI) for Thai male and female of age 19-50 years recommended by Nutrition Division (2003) were also presented in Table 2. It was clearly shown that both seaweeds contained considerably high amount of minerals. Regarding the DRI, both kinds of seaweeds were notably rich in iodine. Apart from iodine, *Caulerpa lentillifera* was also rich in phosphorus, calcium, magnesium and copper while *Ulva reticulata* was rich in potassium, manganese and ferrous. Similar to other edible seaweed, *Gracilaria changgi* was reported to contain high level of calcium (651), zinc (13.8),

Table 2 Mineral contents (mg/100 g dry basis except Cu and I in μg/100g) of *Caulerpa lentillifera*, *Ulva reticulata* and *Gracilaria changgi*[a] compared to Dietary Reference Intake (mg/day except Cu and I in μg/day).

Minerals	*Caulerpa lentillifera*	*Ulva reticulata*	*Gracilaria*[a] *Changgi*	DRI[b] male	DRI[b] female
P	1030	180	nr	700	700
K	970	1540	nr	nr	nr
Ca	780	140	651	800	800
Mg	630	140	nr	310-320	250-260
Zn	2.6	3.3	13.8	13	7
Mn	7.9	48.1	nr	2.3	1.8
Fe	9.3	174.8	95.6	10.4	24.7
Cu (μg)	2200	600	800	900	900
I (μg)	1424	1124	nr	150	150

[a] Norziah and Ching, 2000

[b] Dietary Reference Intake : the amount recommended for consume daily for Thai adult of age19-50 years (Nutrition Division, 2003)

nr not reported

ferrous (95.6) and copper (0.8) (Norziah and Ching, 2000). Based on the result, these seaweeds may serve as food supplements to help meet the recommended daily adult intakes of some minerals.

Vitamins

Quantitative analysis of vitamins was calculated on the basis of 100g fresh edible portion and was presented in Table 3. Comparison study was made on the amount found in the green seaweed samples to those presented in various locally available vegetables. The vitamin A content, presented as total Retinol Equivalent (RE), found in both seaweeds at moderate amount, i.e. about 7 times lower than the vitamin A-rich vegetable such as carrot, but more than twenty times greater than that found in cabbages. *Caulerpa lentillifera* was also considered to be rich in vitamin E with moderate amount of vitamin B_1, vitamin B_2 and Niacin while *Ulva reticulata* was lack of vitamins except vitamin A.

Fatty acid composition

As the total lipids content of seaweeds was quite low so they were not a conventional sources of energy. However, most of them were reported to be rich in polyunsaturated fatty acid regarding to their fatty acid composition (Darcy-Vrillon, 1993). Variations in fatty acid contents are due to both environment and genetic differences mentioned above (Sanchez-Machado *et al*., 2004). In this work, thirteen fatty acids were identified. The fatty acid composition of *Caulerpa lentillifera* and *Ulva reticulata* were shown in Table 4, together with some other seaweeds reported. It was found that the most abudant fatty acid in both seaweeds was C16:0 (palmitic acid), which accounted for 67.83% of all fatty acid for *Caulerpa lentillifera* and 41.53% for *Ulva recticulata*. However, they also contained the essential fatty acids of C18:2(w -6) (linoleic acid), C18:3(w-3) (alpha-linolenic acid), C20:5(w -3) (the eicosanoid precursors), C20:4 (w -6) (arachidonic acid) and C20:5 (w -3) (eicosapentaenoic acid) in rather small amounts. The fatty acid pattern of *Caulerpa lentillifera* was similar to that of *Porphyra sp*. (Table 4) but higher in saturated fatty acid (palmitic and stearic acid) and lower in unsaturated fatty acid except linoleic and linolenic acids. The amount of eicosapentaenoic acid and docosahexaenoic acid in *Caulerpa lentillifera* was

Table 3 Vitamin contents (mg/100 g edible portion except vitamin A) of *Caulerpa lentillifera*, *Ulva reticulata* and some other vegetables[a] compared to Dietary Reference Intake[b] (mg/day except vitamin A in mg/day).

Vitamins	*Caulerpa Ientillifera*	*Ulva reticulata*	Cabbage	Carrot	Lettuce	DRI male	DRI female
Total Vitamin A[c]	170	167	7	1116	393	700	600
Vitamin E	2.22	0	nd	nd	nd	15	15
Vitamin C	1.00	0	23	3	24	90	75
Thiamin	0.05	0.01	0.04	0.04	0.06	1.2	1.1
Riboflavin	0.02	0.13	0.22	0.05	0.18	1.3	1.1
Niacin	1.09	0	2.8	0.8	0.6	16	14

[a] from Nutritive values of Thai foods (Nutrition Division, 2001)

[b] Dietary Reference Intake :- the amount recommended for consume daily for adult of age 19-50 years (Nutrition Division, 2003)

[c] RE (Retinol Equivalent) = 1 microgram (mg) retinol or 6 microgram beta carotene

nd not determined

notably lower than those reported in *Palmaria sp*. and *Gracilaria changgi.*

Amino acid composition

Table 5 showed the amino acid profiles of both seaweeds. Fifteen amino acids were detected and the separation of the amino acids in the samples were reasonably resolved. Data on tryptophan, methionine and cysteine were not included in this work since the amino acids are destroyed during acid hydrolysis. Apart from the excluded amino acids, both seaweed samples contained all the essential amino acids in different proportions. Since cysteine and tyrosine can replace methionine and phenylalanine, respectively, through metabolic processes, two amino acids are combined, i.e. methionine with cysteine and phenylalanine with tyrosine for

Table 4 Fatty acid contents (mg/g sample) and profiles (g/100g fatty acids) of *Caulerpa lentilifera*, *Ulva reticulata* and some edible seaweeds.

Fatty acids	mg/g sample		g/100 g fatty acids				
	Caulerpa lentillifera	*Ulva reticulata*	*Caulerpa lentillifera*	*Ulva reticulata*	*Porphyra*[a] *sp.*	*Palmaria*[a] *sp.*	*Gracillaria*[b] *changgi*
C 16:0 Palmitic acid	8.92	1.43	67.83	41.53	63.19	45.44	22.0
C 16:1 Palmitoleic acid	0.80	0.32	6.08	9.29	6.22	5.26	nr
C 18:0 Stearic acid	1.46	0.92	11.1	26.72	1.23	1.28	nr
C 18:1 (w9) Oleic acid	0.03	0.13	0.23	3.77	6.7	3.13	21.9
C 18:2 (w6) Linoleic acid	0.56	0.14	4.26	4.07	1.17	0.69	nr
C 18:3 (w3) Linolenic acid	0.36	0.19	2.73	5.52	0.23	0.59	nr
C 20:0 Arachidate	0.19	0.11	1.48	3.19	nr	nr	nr
C 20:1 Eicosanoate	0.18	0.06	1.36	1.74	4.7	0.20	nr
C 20:4 (w6) Arachidonic acid	0.11	0.04	0.84	1.16	6.8	1.45	nr
C 20:5 (w3) Eicosapentaenoic acid	0.03	0.03	0.83	0.87	6.03	24.05	33.1
C 22:0 Behanate	0.30	0.03	2.28	0.87	nr	nr	nr
C 22:1 Erucate	0.10	0.003	0.76	0.087	nr	nr	nr
C 22:6 (w3) Docosahexaenoic acid	0.11	0.04	0.83	1.16	nr	nr	12.9

nr not reported
a Sanchez-Machado *et al*,,2004
b Norziah and Ching, 2000

nutritional evaluation. The total amino acids (tryptophan, methionine and cysteine not included) were 12.37 g/100g sample (dry weight) in *Caulerpa lentillifera* and 21.27 g/100g sample in *Ulva reticulata*. From these total, 4.7 and 7.09g amino acid/100 g sample corresponding to 37.99 and 35.12% respectively were made up of essential amino acids. If the three excluded amino acids were detected, the proportions would be higher. The amino acid contents found in this study were in consistent to those reported in literatures. In *Gracilaria changgi*, the amino acid pattern was

Table 5 Amino acid compositions (g/100 g sample dry basis) and profiles (g/100 g amino acids) of *Caulerpa lentillifera* and *Ulva reticulata.*

Amino acids	*Caulerpa lentillifera*		*Ulva reticulata*		Egg[a]	Soya[a]
	g/100 g sample	g/100 g amino acids	g/100 g sample	g/100 g amino acids	g/100 g amino acids	g/100 g amino acids
Essential amino acids						
Threonine	0.79	6.38	1.15	5.41	4.7	4.1
Valine	0.87	7.03	1.34	6.30	6.6	5.2
Lysine	0.82	6.63	1.28	6.02	7.0	6.1
Isoleucine	0.62	5.01	0.90	4.23	5.4	5.1
Leucine	0.99	8.00	1.68	7.90	8.6	7.6
Phenylalanine	0.61	4.93	1.12	5.26	9.3 (+Tyr)	8.4(+Tyr)
Total essential amino acids	4.7	37.99	7.47	35.12	41.6	36.5
Nonessential amino acids						
Aspartic acid	1.43	11.56	2.66	12.50		
Serine	0.76	6.14	1.36	6.39		
Glutamic acid	1.78	14.39	2.76	12.98		
Glycine	0.85	6.87	1.38	6.49		
Arginine	0.87	7.03	1.84	8.65		
Histidine	0.08	0.65	0.23	1.08		
Alanine	0.85	6.87	1.72	8.09		
Tyrosine	0.48	3.88	0.77	3.62		
Proline	0.57	4.61	1.08	5.08		
Total nonessential amino acids	7.67	62.0	13.8	64.88		
Total amino acids	12.37	21.27				

[a] Valerie *et al*., 1999

reported to comparable to that of hen's eggs and the ratio of essential to total amino acids was 0.4 (Norziah and Ching, 2000). Wong and Cheung (2000) reported that *Hypnea japonica*, *Hypnea charoides and Ulva lactuca* contained all essential amino acids which accounted for 42.1- 48.4% of total amino acid contents.

In this study, the total amino acid content of each seaweed sample was comparable to its corresponding crude protein content. Both seaweed samples exhibited similar non-essential amino acid patterns, in which aspartic and glutamic acids constituted a substantial amount of the total amino acids (about 25% of total amino acids). Aspartic and glutamic acids were responsible for the special flavor and taste of the seaweeds (Wong and Cheung, 2000).

The result indicated that *Caulerpa lentillifera* and *Ulva reticulata* proteins were of high quality because the essential amino acids represented almost 40% of total amino acids and the essential amino acids profile were closed to those of egg and soya protein (Valerie *et al*., 1999), except for relative lack of data on tryptophan, methionine and cysteine.

CONCLUSION

The edible green seaweeds, *Caulerpa lentillifera* and *Ulva reticulata*, were analyzed for their nutritional compositions and were then compared to those in several other seaweeds and local vegetables. It was found that the two seaweeds studied appeared to be interesting potential sources of plant food proteins owing to their high protein levels and balanced amino acid profiles. In addition, they also showed the potential of being good sources of mineral supplements. The results of the present study concluded that these seaweeds can provide dietary alternatives due to their nutritional values. Their commercial values can be enhanced by promoting the use in foods and expanding the range of seaweed-based products. Further study needs to be done on the utilization and sensory perceptions of these seaweeds.

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