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Nutritive values and heavy metals of the green seaweed *Halimeda macroloba* Decaisne (Chlorophyta: Halimedaceae) from Phuket

Sureerat Rattanasomboon, Aikkarach Kettawan*, Uthaiwan Suttisansanee, Piya Temviriyanukul

Institute of Nutrition, Mahidol University, Salaya, Nakhon Pathom, 73170, Thailand
*Corresponding author: aikkarach.ket@mahidol.ac.th

Abstract : The aim of this study was to determine nutritional values and heavy metals of *Halimeda macroloba*. The young parts and the old parts of the thallus of freshly collected *Halimeda macroloba* from Phuket (Andaman coast) were analyzed separately for six proximates, five macro minerals and two heavy metals. Young and old thallus parts of *Halimeda macroloba* contained low energy (20.85±5.08–21.75±4.95 kcal/100g) and low fat (0.01±0.02–0.04±0.01 g/100g). It is a rich source of insoluble fiber (3.59±0.06–3.60±0.11 g/100g). *Halimeda macroloba* contains significantly higher calcium in the old segment (3923±288 mg/100g) than in the new segment (2443±510 mg/100g).The heavy metals lead and cadmium were found in concentrations considered to be safe for consumption. *Halimeda macroloba* may have potential as an excellent source of calcium, but calcium bioaccessibility need to be study as well as safety.

Keywords: proximate, mineral, segment, toxicity

Introduction

Seaweed are good source of nutritional and widely consumed as food in Asian countries. Nowadays, consumption seaweed is increasing due to health benefit. Because of it has high minerals as well as, vitamins, fiber whereas contain low energy (MacArtain, 2007). Moreover, in Thailand green seaweed become interesting than the past. The genus *Halimeda* is a common widespread in Thai waters. Today, *many* research supported *Halimeda* as a source of natural antioxidant compound (e.g. Yoshie, 2002; Takeshi, 2005). *Halimeda macroloba* Decaisne is a tropical green alga that can be found throughout the Indo-Pacific including Thailand where it can be found in the Andaman Sea and in the Gulf of Thailand (Pongparadon, 2015). In Thailand it is called Sarai-Bai-Ma-Kud (Kaffir weed), because the alga consists of flattened segments reminiscent of kaffir lime leaves (figure 1). A few studies have reported difference in the nutritional composition of this seaweed which depend on species, season, habitat, environmental, condition and maturity (Renaud, 2006; Manivannan, 2009; Sivaramakrishnan, 2017). The present study focus on difference of nutritive values in the young and old thallus parts and also analyzed heavy metals of this seaweed. Because of seawater can contaminate with heavy metals depend on variety of factor such as water, environmental and sediment (Rermdumri, 2009).It is a general problem that most found in seaweed. Previous study investigated contamination of heavy metals in the seaweeds of Thailand. The concentration of heavy metals were investigated by Atomic Absorption Spectrometry (AAS) for 52 samples. They are reported six heavy metals cadmium, chromium, copper, mercury, lead and zinc were 0.20–1.44, 1–46, 0.9–50.0, 0–0.839, 0–59, 15–377 µg/g dry weight and suggested the seaweed to indicators of heavy metals in the Gulf of Thailand (Lewmanomont, 1984). At the present time seaweed supplement become interested, it is increase risk to get heavy metals from consumptions in several products. The study of dietary cadmium intake for long-term, the result show that it was associated with increased risk of endometrial cancer in postmenopausal women (Akesson, 2008). It is very interesting for using in nutritional field as health promoting food. Thus, the aims of this study were to determine the nutritive values and heavy metals of *Halimeda macroloba*.

Materials and Methods

Raw Materials

Halimeda macroloba were collected from Tangkhen Bay, Phuket Island (Thailand) in November and December 2017. Each sample was randomly collected from different areas of the bay (n=3). The sample around 1,000–1500 gram fresh basis per 1 individual sample. They were cleaned, eliminated some dirty, remove epiphytes (Figure 2) and sand. Then the sample washed thoroughly with tap water until clean. The holdfast of each sample was removed and the rest of the thallus was separated into two sample of respectively, old and new segments (Figure1).Separation new segments part started from the top of thallus until second layer of segment, lower the new segment and hard segments are old segments part. Each sample was blended until homogenous and kept in -20°C.

Analytical methods

Fat, protein, moisture, ash and dietary fiber contents were determined following the standard methods described in Latimer (2016). Fat content was determined by acid digestion and petroleum ether extraction. Protein content was determined by using kjeldahl. Moisture content was determined by using hot air oven (105°C). Ash content was analyzed using a muffle furnace (550°C). Carbohydrate and energy were determined by calculation. Total dietary fiber, soluble dietary fiber and insoluble dietary fiber was determined by Enzymatic-Gravimetric method. Mineral such as calcium, sodium, and potassium was determined by Flame-Atomic Absorption Spectrometry (AAS). Phosphorus was analyzed by gravimetric method, and magnesium was analyzed by ICP-OES. Heavy metals lead and cadmium was determined using closed system technique of wet digestion in foods and Furnace-Atomic Absorption Spectrometry (Dhananivesku, 2003).

Statistical analysis

All the proximate, phosphorus and magnesium data were treated statistically by independent t-test. The minerals (sodium, potassium and calcium) were analyzed by one-way analysis of variance (ANOVA). All data were expressed as mean ± standard deviation in three replicates (*n*=3). All the statistical analysis was carried out using SPSS for Window 19.0 (SPSS, Chicago, IL, USA). One-Way ANOVA was used for determining the significant difference (*P* values < 0.05 and < 0.001) among the means.

Results and Discussion

Proximate composition

The moisture content of present study (table 1) higher than 73.1±1.4 (Gunji, 2007) might be difference of preparation the sample, they wipe the sample with paper towel before minced the sample. While this study, rest the thallus with a sieve before mince. Even though preparation of dry matter are difference such as they was shade dried before kept in an oven, temperature, and time for dried the seaweed. There are general reason of difference data or result. The ash content of previous study (Renaud, 2006) showed *H. opuntia* (86.0±2%) higher amount of ash than *H. macroloba* (74.4±2%).The present study (Table 1) has higher ash content than above study, Conversion to dry matter of the old segments equal to 74.81%.The inorganic compound in *H. macroloba* of present study might be higher than above study.

The protein content in dry matter of Halimeda species from Andaman Coast of India (Sivaramakrishnan, 2007) showed that *H. tuna* and *H. macroloba* respectively, 12.8±1.25% and 14.56±1.76%. And also has study of India (Manivannan, 2009) was observed *H. tuna* and *H. macroloba* respectively, 23.12±0.86% and 28.94±0.68%. The protein content of India seaweed might be high. The study seasonal variation of tropical Australian marine (Renaud, 2006) were found *H. macroloba* and *H. opuntia* respectively, 6.6±4% and 3.2±4% (summer). The data showed *H. macroloba* protein in summer higher than winter (4.6±4%).Not only protein but also has higher ash, soluble fiber and energy than winter season. It was related to the study of seasonal variation of *H. macroloba* at Phuket province in Thailand (Sinutok, 2008), high light in summer season it might increases photosynthesis and growth rate. Therefore, nutritive values might be higher than winter. The reason of environmental of India and Australia are difference. Thus, India might be high protein than Australia. The present study has protein nearby 1.6±0.1% (Gunji, 2007) compared with fresh basis in the table1.

The carbohydrate content of *H. macroloba and H. tuna* are 17.20±0.71% and *H. tuna* 17.12±0.44% (Manivannan, 2009). The present study (table 1), conversion to dry weight carbohydrate of *H. macroloba* are 23.53 % and 18.47% respectively, new segments and old segments. The present study using calculation whereas above study estimated by following the Phenol-sulphuric acid method of Dubois et al.

The fat content of seaweed are present in relatively low (Manivannan, 2009) was observed the maximum of lipid content is *H. tuna* 3.53±0.25% and the minimum lipid concentration *H. macroloba* 0.26±0.07% using extraction method similarly to present study. The result show that present study has lower fat content than above research (Table 1).

The dietary fiber of seaweed are contain large amount of polysaccharide which are not digested by humans *and* mostly compose of insoluble fiber (Jiménez-Escrig & Sánchez-Muniz, 2000; Gómez-Ordóñez, 2010). Previous study was found *H. macroloba* has Total dietary fiber (14.7±1.6) followed by Insoluble dietary fiber (14.3±1.8), soluble fiber (0.4±0.3) g/100 g dry weight (Santoso, 2004). The present study also has higher Insoluble dietary than soluble fiber (Table1). Conversion the present study result equal to Insoluble fiber of old segment and new segments respectively, (17.19–21 g/100g) thus, the new segment has higher insoluble fiber than above study, the old segments is similar to above study.

For the reason of low fat, high protein content and mostly seaweed compose of insoluble fiber. Therefore, the seaweed get the low energy including fiber and excluding fiber (Table1). Compared with raw wakame 100 gram contained energy 45 kcal, raw spirulina contained energy 26 kcal/100 g (USDA, 2018). Usually, the food which composes dietary fiber with low energy and low fat would be useful for body weight control. The most of dietary fiber of *H.macroloba* was insoluble form, so, it would be useful in excretory system of the body, intestinal health and decrease the risk of intestinal cancer (McIntosh, 1993).Total fiber respectively, raw wakame 0.5 g/100 g and raw spirulina 0.4 g/100 g, (USDA, 2018).It is less than 8–10 times of *H. macroloba* (Table 1).

The calcium content *H.macroloba* was very high (Table 1). For the calcium source in plants, collard green (*Brassica oleracea* L.) has highest calcium (232 mg/100g) among vegetables (USDA, 2018). The calcium content of *H.macroloba* was about 11–17 times of collard greens. Thai Recommended Daily Intakes (Thai RDI, 1998) for calcium is 800 mg/day. Thus, the calcium content 100 g *H.macroloba* is about 3–5 times of Thai RDI. It also contains other minerals such as phosphorus, sodium, potassium and magnesium. It is well known that high sodium intake can induce hypertension. The recommendation daily intake of sodium is not more than 2,400 mg. Thus, the sodium contents of *H. macroloba* was low (Table 1).

The comparison between old segments and new segments were evaluated at $P<0.05$. There were no significant difference in the amounts of fat, carbohydrate, total dietary fiber, soluble and insoluble fiber contents between old segments and new segments of *H.macroloba*. The calcium and other minerals sodium, potassium and magnesium (Table 1) in old segments were higher than new segments. It may be because the older parts had more time to calcify and accumulate minerals than younger parts. The previous examined seasonal effects of this seaweed, the result showed that summer sample are high growth rate and increasing photosynthesis (Sinutok, 2008). The heavy metal contents of *H. macroloba* determined in this study is shown in Table 1.There was no significantly difference between new and old segments. The Thai FDA recommendation for lead and cadmium is maximum 1 mg/kg and 3 mg/kg respectively. Therefore, *H. macroloba* from Tangkhen Bay can be considered safe and less than (Thai FDA, 1986)

Conclusion

H. macroloba collected in Tangkhen Bay contained low energy and fat. It is also a rich source of insoluble fiber. *H. macroloba* contained significantly more calcium in the old segments than new segments than in the new segments. It is also safe from high level of heavy metals lead and cadmium. It is very interesting for using in nutritional field as health promoting food. Further studies would evaluate about bioactive compounds, calcium bioaccessibility. For the safety of this seaweed should be study more in other heavy metals and cytotoxicity. Further research on seaweed should be focus on several of season and many place is necessary to analyze and represent this seaweed from Thai water.

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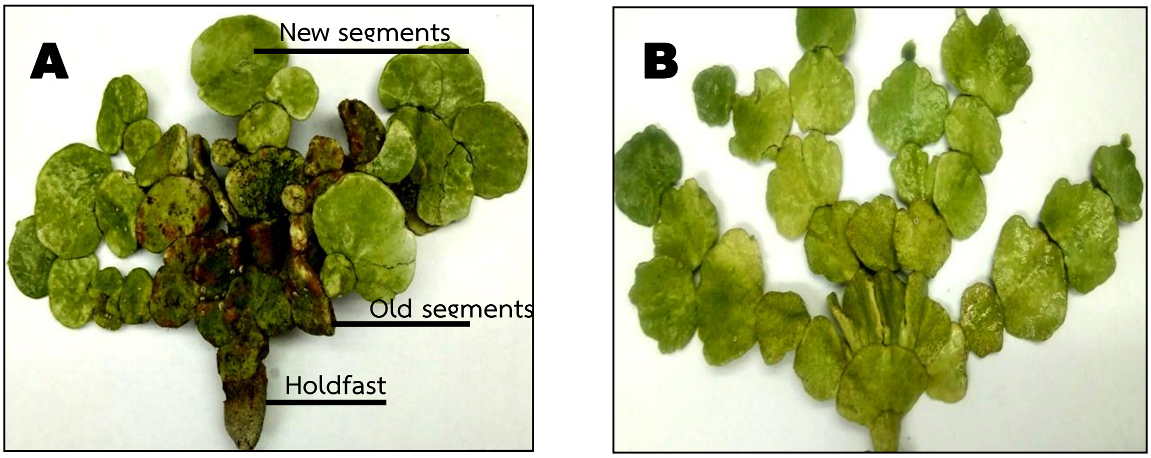

Figure 1 The thallus of *Halimeda macroloba* consist of new segments, old segments and holdfast (root- like structure) before cleaning (A), The thallus of *Halimeda macroloba* after cleaning and removed epiphytes , sand and unwanted impurities which cut the hold fast (B).

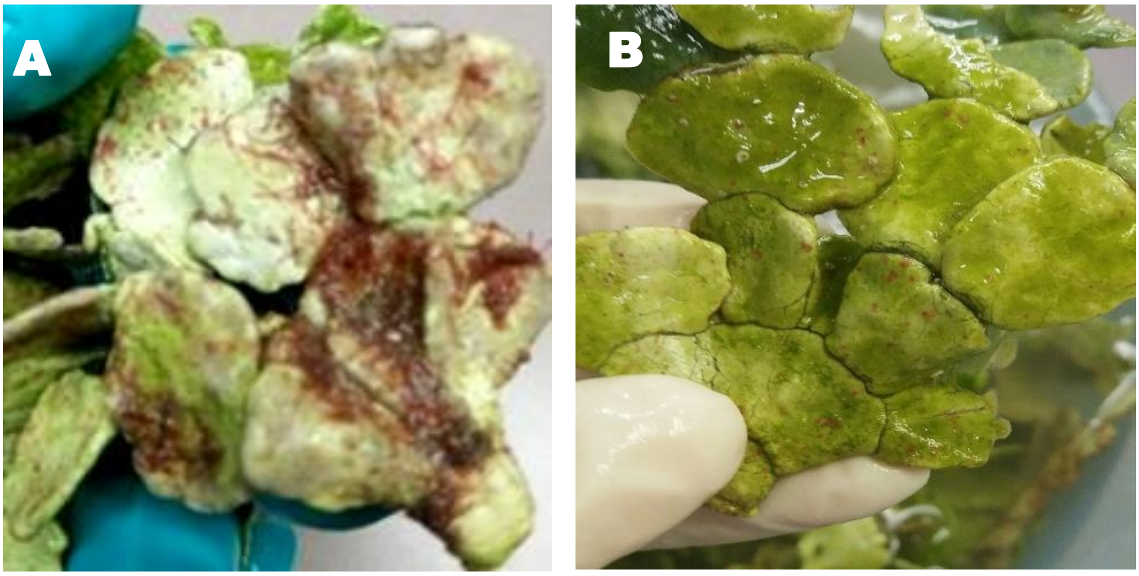

Figure 2 The old segments with epiphytes (A), the old segments after removed and cleaned epiphytes (B).

Table 1 Nutritive values in new segment and old segment of *Halimeda macroloba* (per 100 g fresh basis)

Analysis	New segments	Old segments	*P*-values
Proximate			
Energy(kcal)			
Include fiber	21.75 ± 4.95	20.85 ± 5.08	n.s.
Exclude fiber	5.80 ± 1.32	6.02 ± 1.88	n.s.
Moisture (g)	82.98 ± 2.36	79.14 ± 2.24	n.s.
Protein (g)	1.34 ± 0.04	1.23 ± 0.04	n.s.
Fat (g)	0.01 ± 0.02	0.04 ± 0.01	n.s.
Carbohydrate (g)	4.07 ± 1.23	3.88 ± 1.25	n.s.
Ash (g)	11.54 ± 1.07	15.71 ± 2.79	n.s.
Total fiber (g)	3.74 ± 0.06	3.78 ± 0.12	n.s.
Soluble fiber (g)	0.16 ± 0.00	0.15 ± 0.01	n.s.
Insoluble fiber (g)	3.59 ± 0.06	3.60 ± 0.11	n.s.
Minerals			
Calcium (mg)	2443 ± 510	3923 ± 288	<0.05
Phosphorus (mg)	32.67 ± 0.75	20.27 ± 0.61	n.s.
Sodium (mg)	48.39 ± 7.44	71.39 ± 1.21	<0.001
Potassium (mg)	19.64 ± 1.45	24.98 ± 1.58	n.s.
Magnesium (mg)	12.12 ± 1.42	22.31 ± 4.82	<0.05
Heavy metals (per 1000g)	0.27 ± 0.06	0.28 ± 0.07	n.s.
Lead (mg)	0.03 ± 0.01	0.02 ± 0.01	n.s.
Cadmium (mg)			

Mean±SD (n=3). Values within the same row are significantly different ($p<0.05$ and $p<0.001$).