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Original Article

Nutritional composition and physicochemical properties of two green seaweeds (*Ulva pertusa* and *U. intestinalis*) from the Pattani Bay in Southern Thailand

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Abstract

The chemical composition, amino acid, and element contents, as well as some physicochemical properties of *Ulva pertusa* and *U. intestinalis,* collected from the Pattani Bay in Southern Thailand in the rainy and summer seasons of 2007–2008, were investigated in order to gain more nutritional information. It was found that the two green seaweed species contained high level of protein (14.6–19.5% DW), lipid (2.1–8.7% DW), ash (25.9–28.6% DW), soluble fiber (25.3–39.6% DW), insoluble fiber (21.8–33.5% DW) and total dietary fiber (51.3–62.2% DW). Comparing the element contents of the two species, *U. pertusa* was rich in Mg, K and Ca, while *U. intestinalis* was rich in Mg, K, Cl, Na, and Ca. The essential amino acids of the two species were rich in leucine, valine, and arginine contents. The most limiting essential amino acid of both species was lysine. However, the nutritional composition of the two seaweeds varied depending on seasonal change. As for the physicochemical properties of both seaweeds, their swelling capacity (SWC), water holding capacity (WHC), and oil holding capacity (OHC) ranged from 4.0 to 6.4 ml/g DW, 7.8 to 15.0 g/g DW and 1.4 to 4.8 g oil/g DW, respectively. WHC and OHC of *U. intestinalis* was higher than those of *U. pertusa* ($P<0.05$). This study suggested that both species could be potentially used as raw materials or ingredients to improve the nutritive value and texture of functional food and healthy products for human beings.

Keywords: nutritional composition, physicochemical properties, *Ulva* spp., green seaweeds, marine algae

1. Introduction

Marine macroalgae, commonly referred to seaweeds, are categorized by their pigmentation, morphology, anatomy, and nutritional composition as red (Rhodophyta), brown (Phaeophyta) or green seaweeds (Chlorophyta) (Dawczynski *et al*., 2007). About 250 macroalgal species have been commercially utilized worldwide and about 150 species are favorably consumed as human food (Kumari, 2010). In Asian countries, seaweeds have been utilized since the ancient times as source of human food, animal feed, fertilizer, fungicides, as well as herbicides (Fleurence, 1999; Sánchez-Machado *et al*., 2004). However, in Western countries seaweed polymers are especially used as sources of phycocolloids, thickening, and gelling agents in food and pharmaceutical industries (Mabeau and Fleurence, 1993; Rupérez, 2002). At present, the demand of food supplements from seaweeds has increased in Europe, North America, and South America (Manivannan *et al.*, 2009).

Seaweeds are valuable sources of protein, fiber, vitamins, polyunsaturated fatty acids, macro and trace elements, as well as important bioactive compounds (Ortiz *et al*., 2006). Thus, they have been recognized as being beneficial for human and animal health (Fleurence, 1999). However, the

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nutrient compositions of seaweeds are different depending on species, habitats, maturity and environmental conditions (Ito and Hori, 1989). Generally, green and red seaweeds contain higher protein contents (10–30% DW, dry weight) than brown seaweeds (5–15% DW). Proteins are composed of various amino acids and their nutritional quality can be evaluated against the recommended amino acid pattern (FAO/WHO/UNU, 1985; Wong and Cheung, 2000; Matanjun *et al*., 2009).

The lipid content of marine seaweeds accounts for 1–6% DW and provides a low amount of energy. The fatty acids in seaweeds are composed of polyunsaturated fatty acids (PUFAs) (Dawczynski *et al*., 2007; Kumari *et al.*, 2010). They are good nutritional sources of C18 and C20 PUFAs such as eicosapentanoic acid (EPA) and docosahexaenoic acid (DHA), which cannot be synthesized by human bodies and must be gained from diet only (Kumari *et al.*, 2010).

The element contents in seaweed are interpreted through their ash contents which range between 8–40% DW (Mabeau and Fleurence, 1993). Most seaweeds have more ash contents than terrestrial plants and animal products. Some of the trace elements in seaweeds are rare or absent in terrestrial plants (Ito and Hori, 1989; Rupérez, 2002). Thus, seaweeds are important sources of elements vital for the metabolic reactions in the human and animal health, such as enzymatic regulation of lipid, carbohydrate and protein metabolism (Nisizawa *et al*., 1987; Insel *et al*., 2007).

Seaweeds are also good sources of dietary fiber (33–50% DW), which can be classified as soluble dietary fiber (SDF) and insoluble dietary fiber (IDF) (Lahaye, 1991; Rupérez and Saura-Calixto, 2001). The characteristics of dietary fiber are related to their physicochemical properties such as swelling capacity (SWC), water holding capacity (WHC), and oil holding capacity (OHC), which are important for improvement of functional properties in food (Elleuch *et al.*, 2010).

In the Southern coast of Thailand, especially in the Pattani Bay, twelve seaweed species have been found (Ruangchuay *et al.*, 2007). Red seaweed (*Gracilaria* spp.) and green seaweed (*Ulva* spp.) have been abundant in the coastal area. However, the utilzation of seaweeds is restricted to communities living in the coastal area. *Gracilaria tenuistipitata* and *G. fisheri* have been served fresh or used in dried products for both human beings and animals, whereas *Ulva* species are still under-utilized because in Thailand the knowledge about their nutritional composition is still limited.

Therefore the present study aimed to determine the chemical composition, amino acids, and element contents of *Ulva pertusa* and *U. intestinalis* collected from the Pattani Bay in rainy and summer seasons in order to gain extensive information about their nutritional value. Furthermore, this research also investigated some physicochemical properties in order to evaluate their physiological effects in functional and health food.

2. Materials and Methods

2.1 Samples

Samples of the two seaweed species (*U. pertusa* and *U. intestinalis*) were purchased from fishermen who collected them manually from the coastal area of the Pattani Bay during the rainy season in December 2007 and summer in April 2008. The seaweeds were thoroughly cleaned with running water to remove salt, foreign materials such as epiphyte, sand, shells. All cleaned seaweeds were dried at 60°C in a tray dryer for eight hours. Then they were ground into fine powder that could pass through a 0.5 mm sieve. The powder was dried again in an air oven at 60°C for three hours. Then it was stored in sealed aluminum bags at room temperature for further analysis.

2.2 Methods

2.2.1 Proximate analysis

The chemical compositions (protein, ash, and moisture contents) of the powder samples were determined according to the standard method (AOAC, 2000). The moisture content was determined by oven method at 105°C until their constant weight was obtained. Crude protein content was analyzed by the Kjeldahl method with a conversion factor of 6.25 to convert total nitrogen into crude protein. Ash content was acquired by heating the sample overnight in a furnace at 525°C and the content was determined gravimetrically. Crude lipid was extracted from the seaweed powder in a Soxhlet extractor with chloroform:methanol, 2:1, v/v by the method of Bligh and Dryer (1959). The crude lipid content was measured gravimetrically after oven-drying (80°C) the extract overnight. An enzymatic–gravimetric procedure was used to determine the soluble, insoluble and total dietary fiber (AOAC, 2000).

2.2.2 Macro and trace elements

The element contents of samples were analyzed by atomic absorption spectrophotometry (AAS) for Ca, Mg, K, and Na (method 985.35; AOAC, 2000), by inductively coupled plasma optical emission spectrometry (ICP-OES) for Cu and Zn (method 984.27; AOAC, 2000), by gravimetric method for P (Kolthoff *et al*., 1969) and by Chloride analyzer for Cl.

2.2.3 Amino acid analysis

Amino acids were analyzed by the Waters Associates AccQ Tag method of high performance liquid chromatography (HPLC) (Liu *et al.*, 1995). The samples were hydrolyzed with 6 N HCl containing 1% phenol in a heating block at 110°C for 22 hours in sealed glass tubes under a N_2 atmosphere. The HCl and phenol were then driven off by rotary evaporator. An internal standard was then added into the cooled hydrolysate which was diluted with deionized water and then 10 µL of this filtrate was mixed with 70 µL of AccQ fluor derivatization buffer and 20 µL of AccQ fluor reagent. Samples were then heated at 55°C for 10 min in a heating block, before being cooled and analyzed. The chromatographic separation was performed by using Waters Allicance 2695 HPLC system with Waters 2475 Multi λ fluorescence detector which was set at an excitation wavelength of 250 nm and an emission wavelength of 395 nm. The separation was achieved in a Hypersil Gold C18 column (3.9x 150 mm, particle size 3µm). The mobile phase consisted of two solvents, (A) AccQ·Tag and (B) Acetonitrile in water. The identification of the sample amino acids was carried out by comparing their holding time with the retention times of amino acid standards (Sigma Chemicals).

Essential amino acids was compared with the FAO/ WHO reference amino acid pattern of pre-school children and calculated by the method of FAO/WHO/UNU (1985) as shown below:

$$\text{Amino acid score (\%)} = \frac{\text{mg of amino acid per g of test protein}}{\text{mg of amino acid per g of reference protein}} \cdot 100$$

2.2.4 Physicochemical properties

Swelling capacity (SWC) of seaweed samples was measured by the bed volume technique after being equilibrated in excess solvent (Kuniak and Marchessault, 1972). Water holding capacity (WHC) was measured by the modified centrifugation method described by Suzuki *et al.* (1996). Oil holding capacity (OHC) of samples was determined by the slight modified method of Caprez *et al.* (1986). These properties were studied at room temperature (30±2°C). The results of SWC, WHC, and OHC were expressed as ml/g DW, g/g DW and g oil/g DW, respectively.

2.3 Statistical analysis

All determinations were performed at least in four times. Statistical analysis was carried out by using the SPSS 10.0 version software for windows. The analyzed data were expressed as mean with standard deviation (SD). Paired sample t-test was used to identify significant differences at $P<0.05$ between the mean values of both species and between the mean value of each species in two different seasons (rainy and summer seasons).

3. Results and Discussion

3.1 Chemical composition

3.1.1 Protein

The chemical composition of *U. pertusa* and *U. intestinalis* in the present study is shown in Table 1. The moisture contents of *U. pertusa* and *U. intestinalis* collected in rainy and summer seasons were similar, that is within the range 5.4–7.2% of the dry weight (DW). Both seaweed species contained relatively high protein values. The protein content of *U. intestinalis* (17.9% DW) was significantly higher than that of *U. pertusa* (15.4% DW). This result was consistent with the report of Fleurence (1999) who described that *Ulva* spp. had protein content within the range 10–26% (DW). Also, the two species' protein contents were similar to those of *Enteromorpha intestinalis* (16.4% DW) and *Padina gymnospora* (17.1% DW) (Manivannan *et al.,* 2008) but lower than those of some red seaweed species such as *Porphyra tenera* (47% DW) and *Palmaria palmate* (35% DW) (Fleurence, 1999).

Also, this study showed that the protein contents of *Ulva* species were compared with those of other green seaweeds such as *U. reticulata* (21.06% DW) from the Pattani Bay and *Caulerpa lentillifera* collected from aquaculture ponds in Petchaburi, Thailand (12.5% DW) (Rattana-arporn

Table 1. Chemical composition of dried seaweeds (% dry weight, DW)

Seaweeds	Collection time	Protein	Lipid	Ash	Moisture	TDF
U. pertusa	Rainy season	16.1 ± 0.6^R	7.4 ± 1.0^R	28.6 ± 1.4^R	6.0 ± 0.3	52.2 ± 3.8
	Summer	14.6 ± 0.3	2.1 ± 0.0	25.9 ± 0.1	5.9 ± 0.3	59.0 ± 0.3^S
	Mean ± SD	15.4 ± 0.9	4.8 ± 2.9	27.2 ± 1.7	6.0 ± 0.3	55.6 ± 4.4
U. intestinalis	Rainy season	16.4 ± 0.1	8.7 ± 0.6^R	28.4 ± 0.2^R	5.4 ± 0.9	62.2 ± 2.8^R
	Summer	19.5 ± 0.3^S	7.3 ± 0.3	26.9 ± 0.6	7.2 ± 0.2^S	51.3 ± 0.3
	Mean ± SD	17.9 ± 1.7*	8.0 ± 0.9*	27.6 ± 0.9	6.3 ± 1.1	56.7 ± 6.1

Values are expressed as mean ± SD, n=4. ^{R} Signifies significantly higher in rainy season ($P<0.05$). ^{S} Signifies significantly higher in summer ($P<0.05$). *Signifies significantly higher between two species ($P<0.05$).

and Chirapart, 2006), *Enteromorpha intestinalis* (11.4% DW), *E. compressa* (12.3% DW), *U. reticulata* (13.5% DW) collected from the Southeast coast of India (Manivannan *et al.,* 2009) and *U. fasciata* (12.3% DW) collected from Hawai'ian islands (McDermid and Stercke, 2003). Obviously, variation in the protein contents depends on the species, seasons, and environmental conditions.

3.1.2 Lipid

According to reported literature, most seaweeds contained lipid less than 4% DW (McDermid and Stuercke, 2003). But some seaweeds had high level of crude lipid, such as *Dictyota acutiloba* (16.1% DW) and *D. sandvicenis* (20.2 % DW) (McDermid and Stercke, 2003). From this research it was found that the lipid contents were within the range 2.1–7.4% and 7.3–8.7% DW for *U. pertusa* and *U. intestinalis*, respectively. The results were consistent with previous finding for *Enteromorpha* spp. (7.8% DW), harvested at Rewa from the Gulf of Gdañsk coast on the Southern Baltic Sea (Haroon *et al*., 2000). However, they showed higher level of lipid than other green seaweeds (range 0.75–1.64% DW) such as *Caulerpa lentilifera*, *U. reticulata* and *U. lactuca* (Wong and Cheung, 2000; Rattana-arporn and Chirapart, 2006). The difference could have been due to the methodology of lipid analysis. In this study, the determination of lipid content was modified by using Soxhlet extraction (AOAC, 2000) with the mixture of chloroform and methanol 2:1 (Bligh and Dryer, 1959). This large quality of extracts might be due to the use of different methods or geographical and seasonal factors (Haroon *et al*., 2000).

3.1.3 Ash

Earlier literature described that the different seaweeds consisted of different ash contents between 8 to 40% DW (Mabeau and Fleurence, 1993). In this study, the ash contents of sample species were 27.2% DW for *U. pertusa* and 27.6% DW for *U. intestinalis*. These were similar to those of *E. flexuosa* (23.2% DW), *U. fasciata* (25.4% DW) (McDermid and Stercke, 2003) and *C. lentillifera* (24.2% DW) (Rattana-arporn and Chirapart, 2006). The ash values obtained in the present research were compared with those of other green seaweeds such as *U. lactuca* (21.3% DW) (Wong and Cheung, 2000), *U. reticulata* (17.58% DW) (Rattana-arporn and Chirapart, 2006). The amounts of ash contents obtained in the present study were also in agreement with those in other previous reports (Rupérez, 2002; Sánchez-Machado *et al*., 2004). Furthermore, the ash contents in the two seaweeds were higher than those in terrestrial plants, with an average value of 5–10% DW (USDA, 2001). The variation in ash contents also depends on seaweed species, geographical origins, their method of mineralization, as well as effect of food processing by drying and canning (Nisizawa *et al*., 1987; Sánchez-Machado *et al*., 2004).

3.2 Soluble, insoluble and total dietary fiber

Seaweeds are known as abundant sources of high polysaccharide content which contain high level of soluble and insoluble dietary fiber (Lahaye, 1991). In this study, the amounts of soluble, insoluble, and total dietary fiber of both species ranged from 25.3–39.6% DW, 21.8–33.5% DW and 51.3–62.2% DW, respectively (Table 2). However, no significant differences on soluble dietary fiber (SDF), insoluble dietary fiber (IDF) and total dietary fiber (TDF) were found between two species. These analyzed contents agreed with the values previously studied in *U. lactuca* and *Durvilllaea antarctica* (Ortiz *et al*., 2006). The TDF contents of the studied seaweeds were higher than those determined in terrestrial plants such as whole wheat bran (44.5% DW), beans (36.5% DW), onions (16.9% DW) (Prosky *et al*., 1992; Wong and Cheung, 2001) and *Porphyra* spp. (Lahaye, 1991).

Moreover, the study also found that the ratio of SDF to IDF ranged from 0.8–1.7. From the previous literature reported that the soluble dietary fiber is regarded influential in slower digestion, absorption of nutrients, reduce levels of blood cholesterol and glucose. It also plays an important role

Table 2. Composition of dietary fiber in *U. pertusa* and *U. intestinalis* (% DW)

Seaweeds	Collection time	SDF	IDF	TDF	SDF/IDF
U. pertusa	Rainy season	30.4 ± 3.7^R	21.8 ± 0.4	52.2 ± 3.8	1.4 ± 0.2^R
	Summer	25.3 ± 0.6	33.5 ± 0.3^S	59.0 ± 0.3^S	0.8 ± 0.0
	Mean ± SD	27.8 ± 3.6	27.6 ± 6.2	55.6 ± 4.4	1.1 ± 0.4
U. intestinalis	Rainy season	39.6 ± 0.9^R	22.6 ± 2.6	62.2 ± 2.8^R	1.7 ± 0.2^R
	Summer	25.3 ± 0.3	26.0 ± 0.3^S	51.3 ± 0.3	1.0 ± 0.0
	Mean ± SD	32.4 ± 7.7	24.3 ± 2.5	56.7 ± 6.1	1.4 ± 0.4

Values are expressed as mean ± SD, n=4. ^{R} Signifies significantly higher in rainy season ($P<0.05$). ^{S} Signifies significantly higher in summer ($P<0.05$). *Signifies significantly higher between two species ($P<0.05$).

in preventing constipation, colon cancer, cardiovascular disease and obesity (Ortiz, 2006). In contrast, insoluble dietary fiber is associated with fecal bulk increase and intestinal transit time decrease (Potty, 1996). Thus, two *Ulva* species appeared to be interesting source of raw material or ingredients for producing functional food or health promoting food.

3.3 Element contents

The elements in the two seaweeds are shown in Table 3. The macro elements (Mg, K, Ca, Na, Cl, and P) and trace elements (Cu and Zn) ranged from 117.3–4115.2 mg/100 g DW and 0.6–1.5 mg/100g DW, respectively. Some element contents (K, Ca, Na, Cl, P and Zn) of *U. intestinalis* were found significantly higher than those of *U. pertusa*. However, the result indicated that *U. pertusa* was rich in Mg, K and Ca while *U. intestinalis* was rich in Mg, K, Na and Cl. The Na, Cl, and K are also responsible for the maintenance of body fluid balance (Insel *et al*., 2007). The Na/K ratios in the two *Ulva* species ranged between 0.25–0.55, which is a favorable value and also agreed with the previous reports (Rupérez, 2002; McDermid and Stercke, 2003; Matanjun *et al*., 2009). These findings suggested that the intake of both species can help balance Na/K ratio diets. In contrast, the consumption of foods with a high Na level may relate to the risk of hypertension (Insel *et al*., 2007).

For the trace elements, the sum of copper and zinc contents were found within the range 1.3–2.6 mg/100 g DW which are below the maximum level (10 mg/100 g) allow in Japan and France in seaweeds for human consumption (Indergaard and Minsaas, 1991). The levels of the detected elements fit within the ranges observed in previous reports on seaweeds (Mabeau and Fleurence, 1993; Rupérez, 2002). The results of the present study indicated the possibility of using the two *Ulva* species as food supplements to improve nutritive values of human diet and regulate the body fluid balance. Further studies should analyze their toxic elements (arsenic, lead, cadmium, tin, and mercury) to assess toxicological effects before consumption.

3.4 Amino acid composition

The amino acid contents of the two *Ulva* species are shown in Table 4. They included essential amino acids (EAAs): methionine, leucine, isoleucine, lysine, phenylalanine, tyrosine, arginine, threonine, and valine. For tryptophane and cysteine could not be determined by the methods used. *U. pertusa* was rich in leucine, arginine and valine whereas *U. intestinalis* was rich in leucine, threonine, valine, and arginine. The non-EAAs, which are histidine, aspartic acid, glutamic acid, serine, proline, glycine, and alanine, were present in relatively high levels. The two species contained a large amount of aspartic and glutamic acids which gave the special flavors and tastes. The contents of total amino acid ranged from 9.5–10.6 mg/100 mg DW (613–618 mg/g protein) (Table 4) while the protein content were 15.4% DW to *U. pertusa* and 17.9% DW to *U. intestinalis* (Table 1). Leucine made up the highest amount of EAA in the two species. The ratios of EAA to non-EAA of both seaweeds ranged from 0.67–0.72 and their ratios of EAA to total amino acids were almost 0.4. These results agreed with previous reports for *C. lentillifera*, *U. reticulata* (Rattana-arporn and Chirapat, 2006) and *U. lactuca* (Wong and

Table 3. Elements content of *U. pertusa* and *U. intestinalis*.

Elements	*U. pertusa*			*U. intestinalis*		
	Collection time		Overall Mean ± SD	Collection time		Overall Mean ± SD
	Rainy season	Summer		Rainy season	Summer	
Macro elements(mg/100g DW)						
Mg	3,359.6	3,980.4	3,670.0 ± 533.0	2,018.3	4,115.2^S	3,098.1 ± 1,157.2
K	1,535.7^R	912.5	1,224.1 ± 349.2	2,805.8^R	2,456.8	2,538.6 ± 320.3*
Ca	604.5	734.3^S	669.4 ± 76.2	1,369.4^R	794.5	1,047.8 ± 352.6*
Na	434.9^R	318.5	376.7 ± 63.3	698.7	1,711.9^S	1,064.5 ± 489.1*
Cl	117.3	175.0^S	146.1 ± 32.1	327.4	3094.0^S	1705.5 ± 1474.0*
P	160.2	193.8	177.0 ± 33.2	219.0	455.7	271.9 ± 115.1*
Na/K ratio	0.28	0.35^S	0.32 ± 0.04	0.25	0.55^S	0.44 ± 0.22
Trace elements(mg/100g DW)						
Cu	1.2^R	0.7	1.0 ± 0.3	1.2^R	0.6	0.9 ± 0.3
Zn	1.0^R	0.6	0.8 ± 0.2	1.4	1.5^S	1.5 ± 0.2*

Values are expressed as mean ± SD, n=4. ^{R} Signifies significantly higher in rainy season (*P<0.05*). ^{S} Signifies significantly higher in summer (*P*<0.05). *Signifies significantly higher between two species (*P*<0.05).

Table 4. Amino acid content of *U. pertusa* and *U. intestinalis* for two seasons.

Amino acid	*U. pertusa*		*U. intestinalis*	
	mg/100 mg DW	mg/g protein	mg/100 mg DW	mg/g protein
Aspartic acid[b]	1.19 ± 0.06	77.1 ± 1.4	1.46 ± 0.06*	83.1 ± 5.5
Serine[b]	0.58 ± 0.01	37.7 ± 2.0	0.65 ± 0.03*	36.9 ± 2.2
Glutamic acid[b]	1.17 ± 0.22	75.6 ± 10.0	1.42 ± 0.02	81.9 ± 7.4
Glycine[b]	0.66 ± 0.02	43.0 ± 1.5	0.74 ± 0.01*	42.8 ± 3.7
Histidine[b]	0.13 ± 0.04	8.6 ± 2.8	0.13 ± 0.01	7.4 ± 1.2
Arginine[a]	0.62 ± 0.10	39.9 ± 4.2	0.57 ± 0.03	33.5 ± 4.5
Threonine[a]	0.54 ± 0.04	34.8 ± 0.5	0.72 ± 0.03*	41.7 ± 5.1
Alanine[b]	0.92 ± 0.11	59.7 ± 3.7	1.19 ± 0.04*	69.1 ± 8.1
Proline[b]	0.54 ± 0.01*	35.2 ± 1.3	0.50 ± 0.02	29.4 ± 3.7
Tyrosine[a]	0.35 ± 0.02*	22.9 ± 2.4	0.28 ± 0.01	16.2 ± 1.1
Valine[a]	0.60 ± 0.01	39.1 ± 1.9	0.70 ± 0.02	40.5 ± 3.6
Lysine[a]	0.46 ± 0.10	30.1 ± 7.8	0.37 ± 0.08	19.6 ± 2.7
Isoleucine[a]	0.40 ± 0.02	25.9 ± 0.8	0.43 ± 0.02*	25.3 ± 3.4
Leucine[a]	0.80 ± 0.02	52.0 ± 2.4	0.85 ± 0.05	49.7 ± 7.1
Phenylalanine[a]	0.56 ± 0.02	36.7 ± 1.4	0.62 ± 0.00*	35.9 ± 3.5
Total AA	9.50 ± 0.45	618.2 ± 9.1	10.61 ± 0.18*	612.9 ± 56.7
EAA	3.97 ± 0.09	258.4 ± 9.3	4.25 ± 0.09*	246.1 ± 24.5
Non-EAA	5.53 ± 0.37	359.8 ± 6.0	6.36 ± 0.11*	366.8 ± 32.2
EAA/Non-EAA	0.72 ± 0.03*	0.72 ± 0.03	0.67 ± 0.01	0.67 ± 0.01
EAA/ Total AA	0.42 ± 0.01*	0.42 ± 0.01	0.40 ± 0.00	0.40 ± 0.00

Values are expressed as mean ± SD, n=4. *Signifies significantly higher between two species ($P<0.05$). [a] EAA, Essential amino acid. [b] Non-EAA, Non essential amino acid.

Cheung, 2000).

Furthermore, the levels of EAAs in the two seaweeds were compared with the pre-school children's reference amino acid pattern of FAO/WHO/UNU (1985). The essential amino acid scores of *U. pertusa* and *U. intestinalis* ranged between 51.9–102.4% and 33.7–122.7% (Table 5), with threonine at the highest score. It was also found that the most limiting amino acid of both species was lysine, followed by leucine. According to previous reports, the limiting amino acid of *U. lactuca* was leucine (Wong and Cheung, 2000) while that of *Eucheuma cottonii* and *Caulerpa lentiliferra* was lysine (Matanjun *et al.*, 2009). This study shows that the two *Ulva* species can be used as an alternative source of protein and amino acids for human food and animal feeds.

3.5 Physicochemical properties

Most seaweeds are rich in dietary fiber which determined their hydration properties namely swelling capacity (SWC), water holding capacity (WHC), and oil holding capacity (OHC). The TDF of the two species ranged 51.3–62.2% (Table 1). Their physicochemical properties are shown in Table 6. The SWC and WHC of both species ranged from 4.00–6.42 ml/g DW and 7.78–14.96 g/g DW, respectively, while the WHC of *U. intestinalis* was higher than that of *U. pertusa* ($P<0.05$). The SWC and WHC values obtained were similar to those of *Fucus vesiculosus*, *Chondrus crispus,* and *Porphyra tenera* (Rupérez and Saura-Calixto, 2001), *U. lactuca* and *E. compressa* (Lahaye and Jegou, 1993) and some agricultural by-products (dietary fiber concentrates) (Grigelmo-Miguel and Martin-Belloso, 1999; Elleuch *et al.*, 2010). However, some studies reported that it was difficult to compare the values of WHC in one kind of seaweed with those of other seaweeds or food because they depended on sample preparation, size and porosity of samples, as well as temperature, time, and centrifugation in experimental conditions (Wong and Cheung, 2000; Elleuch *et al.*, 2011).

The SWC and WHC properties of seaweeds are generally related to their characteristic of polysaccharides as well as protein which links to cell wall of polysaccharide (Fleury and Lahaye, 1991; Fleuence, 1999). This study shows the SWC and WHC values with indicated that both seaweeds may be use as functional ingredient to improve physical and structural properties in food products (Grigelmo-Miguel and Martina-Belloso, 1999; Elleuch *et al.*, 2010).

Besides their hydration properties, fiber has oil hold capacity (OHC). In this study, the OHC of *U. intestinalis* (4.62 g oil/g DW), which comparable to that of *U. fasciata* meal (4.52 g oil/g DW) (Carvalho *et al.*, 2009), was higher than that of *U. pertusa* (1.53 g oil/g DW) ($P<0.05$), which comparable to that of *U. lactuca* (1.46–1.68 g oil/g DW) (Yaich *et al.*, 2011).

Table 5. Essential amino acid score of *U. pertusa* and *U. intestinalis.*

Amino acids	*U. pertusa* (mg/g protein)	*U. intestinalis* (mg/g protein)	Reference (mg/g protein)[a]	Score of *U. pertusa*	Score of *U. intestinalis*
Leucine	52	49.7	66	78.8	75.3
Isoleucine	25.9	25.3	28	92.5	90.4
Lysine	30.1	19.5	58	51.9	33.7
Methionine +Cysteine	ND	ND	25	ND	ND
Threonine	34.8	41.7	34	102.4	122.7
Tyrosine + Phenylalanine	59.5	52.0	63	94.5	82.6
Tryptophane	ND	ND	11	ND	ND

[a] Reference amino acid pattern of preschool children (2-5 years) (FAO/WHO/UNU, 1985). ND, not determined

Table 6. Physicochemical properties of *U. pertusa* and *U. intestinalis.*

Seaweeds	Collection time	SWC (ml/g DW)	WHC (g/g DW)	OHC (g oil/g DW)
U. pertusa	Rainy season	4.00 ± 0.55	7.78 ± 0.29	1.65 ± 0.10^{R}
	Summer	4.58 ± 1.10	8.39 ± 0.54	1.42 ± 0.03
	Mean ± SD	4.29 ± 0.86	8.08 ± 0.52	1.53 ± 0.14
U. intestinalis	Rainy season	6.42 ± 0.32^{R}	12.84 ± 0.69	4.41 ± 0.06
	Summer	4.00 ± 0.55	14.96 ± 0.66^{S}	4.83 ± 0.09^{S}
	Mean ± SD	5.21 ± 1.36	13.90 ± 1.29*	4.62 ± 0.24*

Values are expressed as mean ± SD, n=4. ^{R} Signifies significantly higher in rainy season (P<0.05). ^{S} Signifies significantly higher in summer (P<0.05). *Signifies significantly higher between two species (P<0.05).

In other words the range of OHC of two species is quite extensive when compared with other fiber rich food such as peach (1.02–1.11 g oil/g DW) (Grigelmo-Miguel *et al.*, 1999), jack beans (2.3 g oil/g DW) (Betancur-Ancona *et al.*, 2004), as well as orange peel IDF (3.36 g oil/g DW) (Chau and Huang, 2003). The OHC properties of food particles correlated with their surface properties, overall charge density, lipophilic and hydrophilic constituents (Fleury and Lahaye, 1991; Elleuch *et al.*, 2011). Therefore, the two *Ulva* species can be a good choice as stabilizers in formulate food products. Moreover, they can reduce blood lipid level, obesity and coronary heart disease risk.

3.6 Seasonal effect

Many previous reports listed that the variation in the nutrient contents of seaweeds was related to several environmental factors such as water temperature, salinity, light, and nutrients (Marinho-Soriano *et al.*, 2006; Murakami *et al.*, 2011). Most of these environmental parameters can influence the biosynthesis of several nutrients due to seasonal changes in ecological conditions (Lobban *et al.*, 1985).

From this study, *U. pertusa* collected in the rainy season had significantly higher levels in some nutrients such as protein, lipid, ash, SDF, elements (K Na, Cu, and Zn), and amino acids (glutamic acid, arginine, threonine and alanine) (P<0.05). In contrast, they showed high levels of IDF, TDF, elements (Ca and Cl) and amino acids (histidine and lysine) as well as OHC when collected in summer (P<0.05). *U. intestinalis* collected in the rainy season had significantly higher levels of lipid, ash, TDF, SDF, elements (K, Ca and Cu), and SWC. When *U. intestinalis* was collected in summer, it had higher levels of IDF, elements (Mg, Na, Cl and Zn), amino acids (serine and lysine), as well as WHC and OHC properties (P<0.05) (Tables 1, 2, 3, 4 and 6). These findings agreed with those described in previous reports for *Enteromorpha* spp. (Haroon *et al.,* 2000), *Gracilaria cervicormis, Sargassum vulgare* (Marinho-Soriano *et al.*, 2006), *Grateloupia turuturu* (Denis *et al.,* 2010), and *Sargassum horneri* (Murakami *et al.*, 2011).

4. Conclusion

It was found that *U. pertusa* and *U. intestinalis* contained high levels of ash, appreciable protein and dietary fiber contents and relatively high levels of macro elements, essential amino acids and soluble and insoluble dietary fibers. Thus, these two seaweeds can contribute to human and animal nutritional requirements. Their nutritional compositions together with their physicochemical properties suggest that *Ulva* species have a potential food to be functional ingredients in food industry. Moreover, its consumption has a positive effect on health because they can reduce blood lipid level, obesity and risk of coronary heart diseases. Further studies concerned fatty acid, vitamin, non-starch polysaccharide constituents, and toxic elements are necessary to provide more information for safer and more versatile utilization of these seaweeds.

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