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Chemical composition and physicochemical properties of tropical red seaweed, *Gracilaria changii*

Pei Teng Chan, Patricia Matanjun *

Faculty of Food Science and Nutrition, Universiti Malaysia Sabah, Jalan UMS, 88400 Kota Kinabalu, Sabah, Malaysia

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ABSTRACT

A study on the proximate composition, minerals, vitamins, carotenoids, amino acids, fatty acids profiles and some physicochemical properties of freeze dried *Gracilaria changii* was conducted. It was discovered that this seaweed was high in dietary fibre (64.74 ± 0.82%), low in fat (0.30 ± 0.02%) and Na/K ratio (0.12 ± 0.02). The total amino acid content was 91.90 ± 7.70% mainly essential amino acids (55.87 ± 2.15 mg g^{-1}) which were comparable to FAO/WHO requirements. The fatty acid profiles were dominated by the polyunsaturated fatty acids particularly docosahexaenoic (48.36 ± 6.76%) which led to low ω6/ω3, atherogenic, and thrombogenic index. The physicochemical properties of this seaweed namely the water holding and the swelling capacity were comparable to some commercial fibre rich products. This study suggested that *G. changii* could be potentially used as ingredients to improve nutritive value and texture of functional foods for human consumption.

1. Introduction

The increase in consumer demand for high quality food products had led to growth in the use of new technologies and ingredients to produce functional foods. Several factors that influence changes in consumer demand include health concerns such as cholesterol, cancer, and obesity; changes in demographic characteristics such as ethnicity, population and ageing; changes in distribution systems and price and the need for convenience. Food that is termed as 'functional' are foods that can provide health benefits beyond basic nutrition by reducing the risk of chronic diseases and enhancing the ability to manage chronic diseases, thus improving the quality of life (Holdt & Kraan, 2011). The market for functional food is increasing at an annual rate of 15–20% (Viuda-Martos et al., 2010). With the global growth of the functional foods market, researchers have turned to sourcing natural food components to provide preventive and beneficial effects to human health (Holdt & Kraan, 2011).

Seaweeds are known for their richness in polysaccharides, minerals, vitamins and also some bioactive substances such as proteins, lipids and polyphenols (Matanjun, Mohamed, Mustapha, & Muhammad, 2009) which have various biological activities such as hypolipidaemia (Chan, Matanjun, Yasir, & Tan, 2014) properties. This gives seaweeds great potential as a supplement in functional food or for the extraction of compounds (Holdt & Kraan, 2011; Matanjun et al., 2009). Compared to land plants, the chemical composition of seaweeds has not been properly investigated and most of the available information was on temperate seaweeds from Japan (Wong & Cheung, 2000). The chemical composition of seaweeds varies with species, habitats, maturity and environmental conditions. Agar, carrageenan, and alginate are the most common algal polysaccharides extracted from seaweeds. These seaweed polysaccharides have gained attention recently as new sources of dietary fibres and food ingredients because they cannot entirely be digested by human intestinal enzymes and also of their viscous forming and bulking ability (Viuda-Martos et al., 2010).

In Asia, seaweeds are normally consumed fresh or are being utilised for the production of industrially important phycocolloids. The genus *Gracilaria* is mainly used for the production of agar-agar in some Southeast Asian countries. The growing consumer demand for functional foods had urged the interest to further investigate the functionality of seaweeds in functional food industry as well as pharmaceuticals. With the previous findings showing cholesterol lowering properties of *G. changii* (Chan et al., 2014), this seaweed showed potential to serve as ingredients in functional or nutraceutical applications. The simplicity in its culturing techniques and high biomass also increases the commercial availability of this seaweed (Jong, Thien, Yong, Rodrigues, & Yong, 2015). Moreover, under the 10th Malaysia Development Plan, via the National Key Economic Areas (NKEA), the Entry Point Project (EPP 3) in seaweed farming has been identified to be a potential lucrative cash

* Corresponding author.
E-mail address: patsy@ums.edu.my (P. Matanjun).

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crop commodity. The government has allocated a substantial grant scheme for the large scale production of seaweed nationwide. The total production of seaweed is expected to increase from 13,500 metric tonnes in 2010 to 150,000 metric tonnes in 2020 respectively under this mini-estate initiative (ETP, 2013). With the increasing production of seaweed, it will directly increase its abundances and availability.

In recent years, there has been only one report on nutritional potential of tropical red seaweed, *G. changii* from Malaysia (Norziah & Ching, 2000). Hence, the objectives of the present study were to evaluate the chemical composition and physicochemical properties of freeze dried edible red seaweed, *G. changii* from Sarawak, Malaysia.

2. Method and materials

2.1. Sample collection and preparation

The fresh red seaweed, *G. changii*, was collected from the mangrove area of Santubong, Sarawak, Malaysia. A voucher specimen (FSMP 01) of the seaweed was preserved in the Biochemistry Laboratory, Faculty of Food Science and Nutrition, Universiti Malaysia Sabah. The seaweed collected was then cleaned with distilled water to remove epiphytes, sand and debris. The clean seaweed was immediately placed in a freezer (−40 °C) and then freeze-dried in a freeze dryer for 24 h. The dried sample was ground to powder using a waring blender to pass through a 0.85 mm (pore size) screen and stored in a sealed bag in a freezer (−40 °C) for further analysis.

2.2. Proximate composition determination

The proximate compositions of the freeze dried *G. changii* powder were determined (AOAC, 2000; Matanjun et al., 2009). The moisture content was determined by oven method at 105 °C overnight (AOAC 934.01) and the ash content was gravimetrically determined after heating at 550 °C for 24 h in a muffle furnace (AOAC 930.05). The fat content was extracted in a Soxtec system with petroleum ether (AOAC 991.36) and the protein content was determined using Kjeltec system (N × 6.25) (AOAC 2000.11). Crude fibre was determined with successive hydrolysis with 100 °C 0.26 N sulphuric acid (H_2SO_4) and 0.31 N sodium hydroxide (NaOH) for 30 min each in a digital hot plate (AOAC 962.09). The results were expressed in dry weight (DW) basis and all measurements were performed in triplicate.

2.3. Insoluble, soluble, and total dietary fibre determination

The insoluble (IDF), soluble (SDF) and total dietary fibre (TDF) in *G. changii* were determined according to the enzymatic-gravimetric method AOAC 993.19 and 991.42 (AOAC, 2000) as provided by Megazymes (Megazymes International Ireland, Bray, County Wicklow, Ireland) using Fibertec. The TDF content was determined by the sum of IDF and SDF. The results were expressed in DW basis and all measurements were performed in triplicate.

2.4. Total carotenoids and chlorophylls determination

The total carotenoids (TCC), chlorophyll a and b were determined by the method of Kumar, Ramakritinan, and Kumaraguru (2010). Freeze dried *G. changii* powder (3.0 g) was extracted with 75 mL of hexane:acetone:ethanol (2:1:1, v/v) for 1 h at room temperature (RT) (24 °C). The homogenate was filtered using Whatman No.1 filter paper and the supernatant collected was made up to 100 mL with extraction solvent. Next, 25 mL of water was added and shaken vigorously. Separation of the phase took place after 30 min. Two layers were observed, organic (upper layer) and aqueous (lower layer). The absorbance of the organic layer was measured at 470 nm and the TCC was calculated using the following formula (de Carvalho et al., 2012): carotenoid content ($\mu g\ g^{-1}$) = [A × v (mL) × 10^4]/$A^{1\%}$ × w (g), where A = absorbance; v = total extract volume; w = sample weight; $A^{1\%}$ = 2600 (β-carotene extinction coefficient in hexane). For chlorophyll a and b determination, 1.0 g of ground *G. changii* was homogenised with 25 mL of acetone for 1 min, and then the mixture was centrifuged at 1250*g*, 4 °C for 10 min. The chlorophyll a and b were calculated according to the following equation:

$$C_a = 11.75\ A_{662} - 2.350\ A_{645}$$
$$C_b = 18.61\ A_{645} - 3.960\ A_{662}$$

where C_a = chlorophyll a, C_b = chlorophyll b, A_{662} = absorbance at 662 nm, A_{645} = absorbance at 645 nm.

2.5. Vitamin C determination

All the chemical analyses were carried out in dimmed lighting. The vitamin C content of *G. changii* was determined according to Hernández, Lobo, and González (2006). Freeze dried *G. changii* (1 g) was homogenised with 5 mL metaphosphoric acid (MPA) (5% containing 0.01% butylated hydroxytoluene (BHT) using a mortar and pestle. The mixture was then filtered using Whatman No. 1 filter paper and the supernatant was collected. Dithiothreitol (DTT) (2 mL) (40 mm in Trizma buffer, pH 9.0) was added to 1 mL to the supernatant and kept dark at 4 °C for 30 min in order to convert all the L-dehydroascorbic acid (DHAA) to ascorbic acid. Then, the supernatant was filtered through 0.45 μm PTFE syringe filter into an amber vial. The vitamin C content was detected using an Agilent 1200 series high performance liquid chromatography (HPLC), equipped with a degasser, quaternary pump, autosampler, column oven and UV detector. The mobile phase consisted of HPLC grade 2% acetic acid (v/v): acetonitrile (95:5, v/v) with a flow rate of 0.8 mL min^{-1}. Sample or standard volume (10 μL) was injected onto Agilent Zorbax Eclipse XDB C18 column (4.6 × 150 mm, 5 μm, Agilent Technologies, Santa Clara, CA, USA) at 30 °C. Absorbance was measured at 245 nm. The vitamin C content of sample was quantified using an external ascorbic acid standard (Sigma-Aldrich, St. Louis, MO, USA). The stock ascorbic acid solution (0.05 mg mL^{-1}) in MPA was prepared daily; the point of calibration curve was 0.01–0.05 mg mL^{-1} diluted with mobile phase. Identification and quantification of vitamin C were performed by comparing the retention time and area under the curve of the sample with the standard. The results were expressed in mg 100 g^{-1} DW basis and all measurements were performed in triplicate.

2.6. α-tocopherol determination

All the chemical analyses were carried out in dimmed lighting. The α-tocopherol was determined according to Sánchez-Machado, López-Hernández, and Paseiro-Losada (2002) with slight modification. Sample (5 g) and 0.50 g ascorbic acid (to prevent oxidation) were homogenised with 200 mL ethyl acetate: n-hexane (9:1), using a homogeniser. The mixture was then incubated at RT (24 °C) in a shaking incubator for 2 h in darkness. After that, the mixture was filtered using Whatman No.1 filter paper. The filtrate was concentrated under vacuum using a rotary evaporator, thereafter the dried extract was then diluted with 2 mL mobile phase. The detection of α-tocopherol was done using Agilent 1200 series HPLC equipped with a degasser, autosampler, binary pump, column oven and fluorescence (FLD) detector. The mobile phase used was HPLC grade acetonitrile: methanol (70:30, v/v) with a flow rate of 0.8 mL min^{-1}. Sample or standard (10 μL) was injected onto a

reversed phase Chromolith RP-18e column packed with high purity polymeric silica gel (100 × 3 mm, 2 μm, Chromolith Performance, Merck Millipore, Darmstadt, Germany) at 30 °C. The FLD detector was set at excitation (294 nm) and emission (330 nm). The α-tocopherol of sample was quantified using an external α-tocopherol (Sigma-Aldrich, St. Louis, MO, USA) standard. The stock α-tocopherol 1.0 mg mL^{-1} (in HPLC grade methanol) was prepared daily; the point of calibration curve was 0.005–0.10 mg mL^{-1} diluted with mobile phase. Identification and quantification of α-tocopherol was performed by comparing the retention time and area under the curve of the sample with the standard. The results were expressed in μg g^{-1} DW basis and all measurements were performed in triplicate.

2.7. Extraction and fatty acid determination

The fatty acids (FAs) of *G. changii* were determined by gas chromatographic with flame ionisation detector (GC-FID) quantification of their methyl esters (FAMEs) (Yaich et al., 2011). For the extraction of oil from *G. changii*, 1 g of samples was extracted with 90 mL of petroleum ether in a Soxtec system to obtain the oil as well as to determine its fat content. Hexane (1 mL) was added to 0.1 mL of oil samples, and then 1 mL of sodium methoxide solution was added to the oil solution. The mixture was stirred vigorously using a vortex mixer for 10 s and later was allowed to stand for 10 min to separate the clear solution of FAMEs from the cloudy aqueous layer. The upper layer of the FAMEs was collected and was analysed by Hewlett-Packard 5890 series II plus GC-FID fitted with a capillary DB 23 Supelco column (30 m × 0.25 mm × 0.25 μm, Sigma-Aldrich, St. Louis, MO, USA). The injection volume was 1 μL, the temperature column was programmed from 30 to 250 °C with the increase of 1 °C min^{-1}, and the injection and detector temperatures were set at 250 °C and 260 °C respectively with the split ratio of 1:100, and the carrier gas was hydrogen with a flow rate of 1.3 mL min^{-1}. Identification and quantification of FAMEs were accomplished by comparing the retention times of the peaks with those of pure FAME standard (Sigma, USA) analysed under the same conditions. The results were expressed as a percentage of individual FAs in the lipid fraction. The atherogenic (AI), thrombogenic (TI) and unsaturation index (UI) were calculated (Kumar et al., 2011; Kumari, Kumar, Gupta, Reddy, & Jha, 2010). All measurements were performed in triplicate.

2.8. Amino acid determination

The content of amino acids (AAs) in *G. changii* was determined according to Benjama and Masniyom (2011) with some slight modification. The *G. changii* (0.20 g) was weighed in screw cap test tubes to which 5 mL of 6 N hydrochloride acid (HCl) was added. The test tubes were tightly capped and placed in an oven 110 °C for 24 h to allow complete hydrolysis. The hydrolysates were then cooled down to RT. The cooled hydrolysates were quantitatively transferred into 100 mL volumetric flask, with 4 mL of internal standard; DL-2-aminobutyric (AABA) (2.5 mm) (Millipore Merck, Darmstadt, Germany) was added. The solution was then brought to volume with ultrapure water. Approximately 1 mL of the sample solution was filtered through filter paper before filtering again through a 0.2 μm nylon syringe filter. Next, 10 μL filtered solution was collected into a microcentrifuge tube for derivatisation process.

The HPLC (Waters Corporation e2695, Milford, MA, USA) equipped with degasser, autosampler, and fluorescence detector was used to analyse AAs in *G. changii*. The mobile phase used was: (A) AccQ Tag Eluent A (100 mL dilute with 1000 mL of ultrapure water) and (B) HPLC grade acetonitrile (60%). The mobile phase was filtered through 0.45 μm cellulose membrane filters before it was used. All separation was carried out on an AccQ Tag column (3.9 × 150 mm) (Waters Corporation, Milford, MA, USA), with the column temperature set at 36 °C, flow rate 1.0 mL min^{-1}. The fluorescence detector was operated with a 250 nm excitation and a 395 nm emission wavelength and the run time for the analysis was 50 min. The gradient elution was produced by the following concentration changes: 0.5 min 2% B; 15 min 10% B; 19 min 13% B; 32 min 35% B; 34 min 100% B; 38 min 0% B. The quantity of each AA was determined from the peak area of known quantity of AA standard mixture and peak area of individual AA in sample that contained internal standard. The amount of each AA in *G. changii* was expressed as mg g^{-1} DW basis. All the measurements of AAs were performed in triplicate.

The AA contents were then compared with the FAO reference pattern (FAO/WHO/UNU., 1985; Matanjun et al., 2009). The AA score of EAAs was calculated using the following equation:

AA score (%) = (mg EAA in 1 g of test protein)/(mg EAA in 1 g of reference protein) * 100

2.9. Mineral determination

To remove carbon, the freeze dried *G. changii* (1 g) was ignited and incinerated in a muffle furnace at 550 °C for 24 h. The ash was wet with distilled water, and dissolved with 1 mL of HNO_3. The mixture was shaken and filtered using filter paper. The filtrate was used for mineral determination (Yaich et al., 2011). The macro minerals: sodium (Na), potassium (K), magnesium (Mg), and calcium (Ca) and trace minerals: iron (Fe), zinc (Zn), and copper (Cu) were determined using a Hitachi Z-5000 atomic absorption spectrophotometer (AAS) and also an air-acetylene burner was used. Selenium (Se) was determined using Perkin Elmer ELAN 9000 inductive coupled plasma mass spectrometry (ICP-MS). The concentrations of the elements were determined from calibration curves of the standard elements. The results were expressed in mg 100 g^{-1} DW basis and all measurements were performed in triplicate.

2.10. Determination of physicochemical properties

2.10.1. Water swelling capacity

The water swelling capacity (SC) of *G. changii* was determined according to Gómez-Ordóñez, Jiménez-Escrig, and Rupérez (2010). Distilled water (10 mL) was mixed with freeze dried *G. changii* (0.50 g), vigorously stirred in a 10 mL measuring cylinder and was left at RT (24 °C), 37 °C, 60 °C, and 80 °C for 18 h. The swelling volume was measured and expressed as mL of sample occupied g^{-1} DW of sample. All measurements were performed in triplicate.

2.10.2. Water holding capacity

The water holding capacity (WHC) of sample was determined according to Yaich et al. (2011). Freeze dried *G. changii* (0.50 g) was dispersed in 25 mL of distilled water and placed in a pre-weighed centrifuge tubes. Then, the dispersion was stirred and left at RT (24 °C), 37 °C, 60 °C, and 80 °C for 1 h. The mixture was then centrifuged at 3000*g* for 25 min. The supernatant was discarded and the moisture content of sample was determined by dehydration in an oven at 50 °C for 24 h. The results were expressed as g of water bound g^{-1} DW of sample. All measurements were performed in triplicate.

2.10.3. Oil holding capacity

The oil holding capacity (OHC) of freeze dried *G. changii* was determined according to Yaich et al. (2011). The sample (0.5 g) was mixed with 20 mL of corn oil in a pre-weighed centrifuge tube. The mixture was then stirred and left at RT (24 °C), 37 °C, 60 °C,

and 80 °C concurrently for 1 h. After that, the mixture was centrifuged at 3000 g for 25 min. The oil supernatant was removed and measured. The results were expressed as g oil held g^{-1} DW of sample. The density of oil = 0.92 g mL^{-1}. All measurements were performed in triplicate.

2.11. Statistical analysis

The values were expressed as mean ± standard error (SEM). One way variance analysis (ANOVA) and Duncan test were used to compare the effect of temperature on the physicochemical properties using SPSS system version 17.0 for Windows. A significant difference was considered at the levels of $p < 0.05$.

3. Results and discussions

3.1. Proximate composition

The proximate composition of ground freeze dried *G. changii* is shown in Table 1. The moisture content of freeze dried *G. changii* was 5.32 ± 0.10% DW. The ash content was higher than the value previously reported (22.7%) (Norziah & Ching, 2000). The ash content was in accordance with the previous results reported on ash content of different genus of seaweeds collected from Sabah's (18–40% DW) (Krishmaiah, Sarbatly, Prasad, & Bono, 2008). It was worth noting that the ash content of *G. changii* was much higher than that of land plants (5–10%) (Rupérez, 2002). A high ash content of *G. changii* in the current study indicates the presence of appreciable amounts of diverse minerals.

The protein content of *G. changii* was higher than the previous data (Norziah & Ching, 2000). The protein content of red and green algae (10–47%) was higher than that of brown algae (3–16% DW), and is also higher than other high protein legumes such as soybean (Bocanegra, Bastida, Benedí, Ródenas, & Sánchez-Muniz, 2009). The protein content in *G. changii* was also higher than in other *Gracilaria* species (6.2–11.6%). It was also higher than other seaweed species (Benjama & Masniyom, 2012; Gressler et al., 2010; Hong, Hien, & Son, 2007; Matanjun et al., 2009). The high protein of *G. changii* indicated that this seaweed may become a potential source of protein.

In general, seaweeds exhibit low lipid content (1–3% DW) (Mabeau & Fleurence, 1993). The fat content of *G. changii* was found to be relatively low when compared to the previous data (3.3%) (Norziah & Ching, 2000). Its value was also lower than other *Gracilaria* species collected from India (Gómez-Ordóñez et al., 2010; Kumar et al., 2011). The crude fibre of *G. changii* was higher than previously reported data (24.7%) (Norziah & Ching, 2000) and was 2 times higher than some seaweed species collected from several areas (Hong et al., 2007; Matanjun et al., 2009).

Table 1
The proximate composition of *G. changii*.

Compositions	%DW
Moisture	5.32 ± 0.10
Ash	40.30 ± 0.81
Protein	12.57 ± 1.31
Fat	0.30 ± 0.02
Crude Fibre	29.44 ± 0.30
Carbohydrate	41.52 ± 0.45
Total dietary fibre	64.74 ± 0.82
Soluble fibre	46.71 ± 0.33
Insoluble fibre	18.03 ± 0.92

Values are Mean ± SEM, n = 3.
Dry weight (DW).

3.2. Insoluble, soluble and total dietary fibre content

According to Table 1, the TDF, SDF and IDF of *G. changii* were in accordance with previous results with TDF ranging from 32% to 71% DW, of which SDF constitutes 7–42% DW and IDF ranges from 5% to 29% DW (Mabeau & Fleurence, 1993). The ratio of SDF:TDF and IDF:TDF was 72.15% and 27.85% respectively. This result indicates that the DFs in *G. changii* were mainly composed of SDF, and this differs from land plants. This was in accordance with the data reported previously whereby the SDF:TDF of red seaweed ranged from 39.0 to 80.0% due to their high SDF content (Gómez-Ordóñez et al., 2010; Matanjun et al., 2009). It is generally accepted that fibre sources suitable for use as a food ingredient should have an SDF/IDF ratio close to 1:2 (Figuerola, Hurtado, Estevez, Chiffelle, & Asenjo, 2005). Therefore, with the ratio of SDF/IDF (1:2.5), *G. changii* may have the potential to be used as a functional ingredient in the food industry. Moreover, it was observed that the TDF content of the studied seaweed was higher than the high fibre foods such as barley, wheat germ, flaxseed and also some fibre rich supplements for the treatment of constipation and as slendering diets (Dhingra, Michael, Rajput, & Patil, 2011).

From a nutritional point of view, both SDF and IDF are known to have physiological properties for the prevention and treatment of some diseases such as CVD, obesity, and diabetes. Viscous soluble polysaccharides have been correlated with hypocholesterolaemic and hypoglycaemic effects, whereas insoluble polysaccharides are mainly associated with a decrease in digestive tract transit time (Chan et al., 2014). The average urban Malaysian diet contains only 13–16 g of TDF and this intake value was far from satisfactory as compared to the recommended nutrient intake of 20–30 g/day for humans in order to be considered as "healthy eating" (Ng, 1997). With a daily intake of 20 g *G. changii*, it is able to provide about 50% of the recommended nutrient intakes required. Thus, *G. changii* may become a potential source of DFs to be incorporated into human daily diet.

3.3. Total carotenoids and chlorophylls content

The TCC, chlorophyll a and b in *G. changii* are shown in Table 2. The value of TCC was higher than other red seaweeds reported previously (Hong et al., 2007). The chlorophyll a in *G. changii* was higher than chlorophyll b. This was in agreement with the study done by Hong et al. (2007) which reported that chlorophyll a and b of red seaweeds range from 68 to 162 μg g^{-1} and 25 to 46 μg g^{-1} respectively. The carotenoid compounds, that are mainly present in red seaweeds, such as *Gracilaria* species, may be β-carotene, zeaxanthin, lutein and antheraxanthin (Christaki, Bonos, Giannenas, & Florou-Paneri, 2012). Algal carotenoids especially β-carotene is preferred by the market of natural products, because it is a mixture of trans and cis isomers which may have anticancer activity. Furthermore, this mixture is rarely obtained for synthetic carotenoids. Natural β-carotene is absorbed 10 times more easily by the body compared to the synthetic form (Christaki et al., 2012).

Table 2
The contents of total carotenoids, chlorophylls, vitamin C, and α-tocopherol of *G. changii*.

Compositions	*G. changii*
Vitamin C (mg 100 g^{-1}DW)	2.51 ± 0.20
α-Tocopherol (μg g^{-1}DW)	4.61 ± 0.29
Total carotenoids content (μg g^{-1} DW)	74.22 ± 0.48
Chlorophyll a (μg g^{-1} DW)	577.89 ± 1.07
Chlorophyll b (μg g^{-1} DW)	1.11 ± 0.21

Values are Mean ± SEM, n = 3.
Dry weight (DW).

3.4. Vitamin C and α-tocopherol content

Vitamin C level in green and brown algae ranges between 50 and 300 mg 100 g^{-1} DM, which is comparable to the concentration in parsley and peppers, while values in red algae range from 10 to 80 mg 100 g^{-1} DM (Bocanegra et al., 2009). According to Table 2, the vitamin C content of *G. changii* was lower than previously reported data (Norziah & Ching, 2000). However, the value was comparable to other seaweeds species (Hong et al., 2007). Generally, green, red, and sublittoral brown algae contain low amounts of tocopherols, apparently mostly α-tocopherol (Pires-Cavalcante, de Alencar, de Sousa, Sampaio, & Saker-Sampaio, 2011). The alpha-tocopherol content of *G. changii* is shown in Table 2, its α-tocopherol levels were lower than some *Gracilaria* species (24–77 μg g^{-1}) (Pires-Cavalcante et al., 2011). The variation of the α-tocopherol content may be due to several factors; examples are seasonality, environmental temperature, salinity, methods of preservation and processing.

3.5. Fatty acid profiles

The FA profile of seaweeds differs from that of terrestrial plants whereby seaweeds have a higher proportion of saturated and unsaturated FAs (Bocanegra et al., 2009). Although seaweeds have low lipid contents, the PUFA contents are superior to those of terrestrial vegetables. Red algae are particularly rich in C18 and C20 PUFAs such as EPA (20:5 ω-3) and arachidonic acid (20:4 ω-6) which have nutritional applications that lead to extensive studies for biotechnological, food, feed, cosmetic and pharmaceutical applications (Bocanegra et al., 2009; Kumari et al., 2010).

The FA profile of freeze dried *G. changii* is presented in Table 3. *G. changii* powder had higher proportions of PUFAs > monounsaturated fatty acids (MUFAs) > saturated fatty acids (SFAs). This showed that *G. changii* has a high composition of unsaturated FAs, representing almost 90% of total FAs. The main SFAs present in *G. changii* are palmitic acid (C16:0) and stearic acid (C18:0) and both of these FAs represent 81% of the total SFA. Similar findings were reported in other *Gracilaria* species, the sum of C16:0 and C18:0 represent 85–94% of total SFA (Kumari et al., 2010).

The PUFAs in *G. changii* range from 0.17 ± 0.02 to 48.36 ± 6.76% with the sum of PUFAs of 51.20 ± 6.78%. This value suggested that more than half of the total FAs detected were of PUFAs. The proportion of docosahexaenoic (C22:6ω3) (DHA) representing 94.45% of the total PUFAs indicates that most PUFA detected was of DHA. Apparently there was no EPA (C20:5ω3) detected in *G. changii*. This was in contrast to the previous studies reported where the dominating PUFAs in Rhodophyta was mainly EPA, with DHA as the second main PUFAs (Kumari et al., 2010; Matanjun et al., 2009; Norziah & Ching, 2000; Schmid, Guihéneuf, & Stengel, 2013). Fatty acid, EPA was unable to be traced in *G. changii*. This may be attributed to a relatively untraceable amount in this seaweed that they were beyond the detection limit. The variation in the FA content may also be due to the season of collection as well as other abiotic factors such as light, salinity, and nutrients (Schmid et al., 2013).

As discussed earlier, *G. changii* contains high amounts of PUFAs as compared to MUFAs and SFAs. Omega-3 PUFAs help prevent the growth of atherosclerotic plaque that affects blood clotting, blood pressure and improves the immune function, while ω-6 PUFAs decrease low density lipoprotein cholesterol (LDL-C) and may also decrease high density lipoprotein cholesterol (HDL-C) which adversely induces heart disease risk. Therefore, it is important to maintain a balanced consumption of ω-6 and ω-3 in diet based on a ratio of ω-6/ω-3 < 10 recommended by the WHO (Matanjun et al., 2009). In the current study, the ω-3 PUFAs have a higher value of 98% of the total PUFAs as compared to ω-6 PUFAs having only 2%. This in turn leads to a low ω-6/ω-3 ratio which is within the WHO standard. The low ω-6/ω-3 ratio in *G. changii* might be due to the presence of a high concentration of DHA. Even though, no EPA was detected in *G. changii*, this seaweed contains a high amount of DHA. This suggests that *G. changii* might be a potential food or supplement source to improve the ω-3 deficiency, especially DHA as compared to others *Gracilaria* species. Previous study had shown the beneficial effects of DHA on immune functions (Tomobe et al., 2000). Hence, *G. changii* may be used to compliment diet containing sufficient EPA but low in DHA.

The FA compositions of the dietary fats, particularly of some individual FA, are of great importance in human nutrition and health concern. Low intake of saturated fat and increased PUFAs to SFAs ratio are associated with a lower risk of human coronary heart diseases (Kumar et al., 2011). Thus, the PUFA /SFA ratio is one of the parameters used to assess the nutritional quality of the lipid fraction of foods. In the present study, the PUFA/SAF ratio

Table 3
The fatty acid profiles of *G. changii*.

Fatty acids	% total fatty acids
SFAs	
C4:0	0.04 ± 0.02
C8:0	0.11 ± 0.03
C10:0	0.04 ± 0.04
C11:0	ND
C12:0	0.18 ± 0.05
C13:0	ND
C14:0	0.96 ± 0.15
C15:0	0.09 ± 0.01
C16:0	4.28 ± 0.58
C17:0	0.02 ± 0.01
C18:0	1.82 ± 0.27
MUFAs	
C14:1	ND
C15:1	0.03 ± 0.01
C16:1	0.28 ± 0.04
C17:1	ND
C18:1ω9 (trans)	2.87 ± 0.36
C18:1ω9 (cis)	0.15 ± 0.07
C20:1ω9	ND
C22:1ω9	2.83 ± 0.86
C24:1	32.16 ± 6.78
PUFAs	
C18:2ω6 (trans)	ND
C18:2ω6 (cis)	0.59 ± 0.08
C18:3ω6	0.17 ± 0.02
C18:3ω3	ND
C20:2	ND
C20:3ω3	1.81 ± 0.22
C20:3ω6	ND
C20:4ω6	0.26 ± 0.02
C20:5ω3	ND
C22:2	ND
C22:6ω3	48.36 ± 6.76
Σ SFAs	7.53 ± 1.72
Σ MUFAs	38.30 ± 7.20
Σ PUFAs	51.20 ± 6.78
Σ PUFAs/Σ SFA	6.96 ± 0.98
ω-6/ω-3	0.02 ± 0.00
AI[a]	0.03 ± 0.003
TI[b]	0.04 ± 0.01
UI[c]	368.68 ± 20.01

Values are Mean ± SEM, n = 3.

[a] AI: atherogenic index = (C12:0 + C14:0 + C16:0)/(ω-3PUFAs + ω-6PUFAs + MUFAs).

[b] TI: thrombogenic index = (C14:0 + C16:0 + C18:0) / (0.5 ω-6PUFAs + 3PUFAs + ω-3PUFAs/ω-6PUFAs).

[c] UI: multiply the% of individual FA by the number of double bonds, followed by summing up these contributions (Kumari et al., 2010). Not detected (ND); Omega (ω); Carbon (C); Fatty acids (FAs); Saturated fatty acids (SFAs); Monounsaturated fatty acids (MUFAs); Polyunsaturated fatty acids (PUFAs).

was 6.96 ± 0.98, which is within the nutritional guidelines that recommended a PUFA/SFA ratio above 0.4 (Kumar et al., 2011). Other than PUFA/SFA ratio, the AI and TI are also related to nutritional factors linked with coronary diseases, which are also used to assess the FA nutritional quality. The AI indicates the relationship between the sum of the main saturated FAs and that of the main class of unsaturated FAs, while TI shows the relationship between the pro-thrombogenic and the anti-thrombogenic of FAs as thrombosis is a central event in atherosclerosis (Ghaeni, Ghahfarokhi, & Zaheri, 2013). Therefore, lower AI and TI maintain better nutritional quality of the FAs. The AI and TI of *G. changii*, in the current study was 0.03 ± 0.003 and 0.04 ± 0.007 respectively and these values were lower than other Rhodophyta (AI: 0.45–2.87, TI: 0.52–5.75) previously reported (Kumar et al., 2011). In addition, this TI value was also lower than products such as lamb (1.58), bovine meat (1.08), lean pork (1.37) and milk based products (2.1). In view of this, the addition of seaweeds to meat products, may not only be useful for technological reasons (gel forming) but also could be of a more satisfactory strategy for the development of healthier lipid formulation (Kumar et al., 2011). The meat system with the addition of seaweeds caused an increased in ω-3 PUFAs and decrease in the ω-6/ω-3 PUFAs ratio, as well as the TI index (López-López et al., 2009).

3.6. Amino acid profiles

The AA composition of *G. changii* is presented in Table 4. The total content of AAs in *G. changii* was 91.90 ± 7.70 mg g^{-1} DW (9.19%). This value was comparable to its corresponding crude protein content (12.57%), indicating that the amount of non-protein nitrogenous materials in this seaweed was insignificant. This was in agreement with some brown and red seaweed reported previously (Wong & Cheung, 2000). Nine EAAs including arginine, threonine, tyrosine, valine, methionine, lysine, isoleucine, leucine, phenylalanine and seven non-EAAs (NEAAs) including aspartic acid, glutamic acid, serine, glycine, histidine, alanine, and proline were present in *G. changii* at different proportions, except for tryptophan and cysteine which were eliminated after acid hydrolysis of the protein samples.

The EAAs of *G. changii* ranged from 2.02 ± 0.30–6.59 ± 0.66 mg g^{-1} DW. The EAA/total AA ratio suggests that more than 50% of the AAs were EAAs. This value was higher than the previously reported data (Norziah & Ching, 2000), but comparable to other *Gracilaria* species reported previously (Benjama & Masniyom, 2012; Gressler et al., 2010). The result also indicates a good ratio of EAAs to NEAAs (1.61 ± 0.20). It was discovered that, arginine was found to be the highest EAA in *G. changii*, representing 18.69% of the total AAs and 30.75% of total EAAs. This EAA also termed as 'conditionally EAA'. This EAA found in *G. changii* was important to certain conditions such as during growth, illness, and metabolic stress, as well as during the first month of a new born (Saini, Badole, & Zanwar, 2013). The second highest EAA presence was leucine; representing 18.42% of the total EAAs. The third highest EAA presence was threonine > lysine > isoleucine > phenylalanine > valine > methionine.

For NEAAs, *G. changii* showed high amounts of aspartic and glutamic acids and these two AAs accounted for 18.53% of the total AAs. This was within the range of *Gracilaria* species reported earlier (15–28% of total AAs) (Gressler et al., 2010; Norziah & Ching, 2000). Aspartic and glutamic acid were responsible for the special flavour and taste of seaweeds (Matanjun et al., 2009). The next highest NEAAs were alanine > serine > histidine > glycine > proline.

The AA scores evaluate the actual abundance of individual EAA in a food material and relate it to dietary requirements or a reference protein. The reference pattern used closely reflects the amounts and proportion of AA that the human needs. The AA scoring pattern that uses the pattern of AAs required by children aged 1–3 years provides that the protein needed meets the AA needs of growing pre-school aged children. It should also meet the needs of almost all other age segments of the population (Insel, Ross, McMahon, & Bernstein, 2011). Since cysteine and tyrosine can replace methionine and phenylalanine respectively, through

Table 4
AA content and AA score of *G. changii*.

Amino acids	mg g^{-1} DW	mg g^{-1} protein	Reference protein (mg g^{-1} protein)[d]	AA score (%)
Aspartic acid	8.61 ± 2.27			
Serine	5.24 ± 0.20			
Glutamic acid	8.42 ± 2.20			
Glycine	3.20 ± 1.63			
Histidine	3.43 ± 0.88			
Arginine[a]	17.18 ± 0.84			
Threonine[a]	6.12 ± 0.29	49.34 ± 5.05	34	145.11
Alanine	5.38 ± 0.66			
Proline	1.74 ± 1.01			
Tyrosine[a]	2.94 ± 0.37			
Valine[a]	4.82 ± 0.44	39.10 ± 5.94	35	111.71
Methionine[ac]	2.02 ± 0.30	16.11 ± 2.06	25	64.44
Lysine[a]	6.03 ± 0.31	48.66 ± 5.20	58	83.90
Isoleucine[a]	5.17 ± 0.32	42.26 ± 7.12	28	150.93
Leucine[a]	6.59 ± 0.66	53.35 ± 7.94	66	80.83
Phenylalanine[ab]	5.01 ± 0.50	63.46 ± 6.51	63	100.73
Tryptophan	ND			
Total AAs	91.90 ± 7.70			
Total EAAs	55.87 ± 2.15	452.07 ± 54.26	309	
Total NEAAs	36.02 ± 5.55			
EAAs/total AAs	0.61 ± 0.03			
EAAs/NEAAs	1.61 ± 0.20			

Values are Mean ± SEM, n = 3.
Not detected (ND); Dry weight (DW); Amino acids (AAs); Essential amino acids (EAAs); Non-essential amino acids (NEAAs).
[a] Essential amino acid.
[b] Tyrosine + phenylalanine.
[c] Methionine + cysteine.
[d] Matanjun et al. (2009).

metabolic process, two AAs are combined; that is methionine with cysteine and phenylalanine with tyrosine for calculation of the AA scores. The AA score of *G. changii* ranged from 64.44 to 150.93% as shown in Table 4. All the EAAs were above their respective EAA FAO/WHO requirement pattern. Methionine was the most limiting AA found in *G. changii*, as this AA had the lowest AA score. The second limiting AA was leucine < lysine < phenylalanine < valine < threonine < isoleucine. This was in contrast to previous report that the most limiting AAs in some *Gracilaria* species were lysine and threonine (Benjama & Masniyom, 2012; Norziah & Ching, 2000). With respect to the total EAA (309 mg g^{-1} protein) requirement by FAO/WHO requirement pattern, *G. changii* was considered to be able to contribute adequate levels of total EAAs, implying that the EAAs present in *G. changii* have a high biological protein value.

3.7. Mineral content

The mineral content of *G. changii* is shown in Table 5. Among the macro minerals, K and Na were the most abundant in *G. changii*. The K concentration was 8 times higher than its Na concentration. Benjama and Masniyom (2012) also reported similar results showing *G. fisheri* and *G. tenuistipitata* collected during summer had higher K levels than Na. This was in agreement with previous study where all the Rhodophycean species had K concentration ranging from 6.04 to 21.95% and its Na levels ranging from 1.77 to 8.04% (Kumar et al., 2011; Matanjun et al., 2009). It is worth noting that the ratio of Na/K in *G. changii* was 0.12 ± 0.02. This ratio was lower than other seaweeds species previously reported (Kumar et al., 2011; Matanjun et al., 2009). The intake of high Na/K ratios has been related to the higher incidence of hypertension. Thus, with low Na/K ratio of *G. changii*, it can reduce fluid retention and high blood pressure without the risk of upsetting the K balance. The trace minerals of *G. changii* ranged from 0.62 ± 0.07 to 49.77 ± 0.20 mg 100 g^{-1} DW. Among the trace minerals present, Fe was the most abundant followed by Zn > Cu > Se. The Fe concentration was found higher than other red and green seaweed species (2.61–44.54 mg 100 g^{-1} DW) reported by Kumar et al. (2011) and Matanjun et al. (2009).

Table 5
Mineral content in *G. changii*.

Minerals	mg 100 g^{-1} DW
Na	2118.19 ± 258.11
K	17613.62 ± 207.33
Mg	436.13 ± 7.35
Ca	625.92 ± 32.18
Fe	49.77 ± 0.20
Zn	3.31 ± 0.00
Cu	1.01 ± 0.02
Se	0.62 ± 0.07
Na/K	0.12 ± 0.02

Values are Mean ± SEM, n = 3.
Dry weight (DW); Sodium (Na); Potassium (K); Magnesium (Mg); Calcium (Ca); Iron (Fe); Zinc (Zn); Copper (Cu); Selenium (Se).

The high mineral content of seaweeds is related to their capacity to retain inorganic marine substances, due to the characteristics of their cell surface polysaccharides (Bocanegra et al., 2009). The ability of seaweeds to accumulate metals will depend on a variety of factors such as location, wave, exposure, temperature, salinity, light, pH, nitrogen availability, season, age of the plant, metabolic processes and the affinity of the plant for each element among others (Sánchez-Rodríguez, Huerta-Diaz, Choumiline, Holguín-Quiñones, & Zertuche-González, 2001). The uptake capacity for the trace metals in algae generally occurs in two directions; foremost is the surface reaction which is independent of factors influencing metabolism such as temperature, light, pH, or age of the plants, which seems to be the main uptake mechanism for Zn, while the second is a slower active uptake in which metal ions, like Cu, manganese (Mn), Se, and nickel (Ni) are transported across the cell membrane into the cytoplasm. These uptake processes are directly dependent on metabolic processes and also vary with changes in temperature, light, or age of the plant (Kumar et al., 2011). The presence of high mineral content in *G. changii* may be due to its ability to directly absorb elements from the surrounding seawater. This characteristic was attributed to sulphated polysaccharides present in the cellular wall of the alga. The hydroxyl, sulphate and carboxyl groups of polysaccharides were ion exchangers; therefore they were important sites of complexation of the metallic cation (Yaich et al., 2011). *G. changii* contains adequate amount of minerals which suggested that this seaweed can be utilised as a superior source of mineral supplements, compared with land plants in which the compositions can interfere with mineral uptake by the body.

3.8. Physicochemical properties

The physiological effects of DFs are related to their physicochemical properties. The water associated with fibre is an important consideration when studying the effects of fibre in the diet as water will influence the metabolic activity of fibre along the human gut (Gómez-Ordóñez et al., 2010). The SC and WHC properties of seaweeds are generally related to their characteristic of polysaccharides as well as protein which links to the cell wall of polysaccharide (Benjama & Masniyom, 2011). In this study, the sum of protein and TDF content of *G. changii* was up to 77.31% (Table 1), so the physicochemical properties of the seaweed might be mainly determined by these two chemical components.

The SC, WHC and OHC of *G. changii* are presented in Table 6. In general, as temperature increased, the SC and WHC of *G. changii* powder were also significantly increased, due to the increase in the solubility of the fibres and protein presence in *G. changii*. In this study, the WHC at 24 °C was 6.15 ± 0.06 g g^{-1} DW, it was significantly increased by 116.26% reaching 13.30 ± 0.08 g g^{-1} DW at 80 °C. At 24 °C, the WHC was not only similar to that of *G. fisheri* (5.53 g g^{-1} DW) as reported by Benjama and Masniyom (2012) and Yaich et al. (2011) but also comparable to that of some agricultural by-products such as oat bran (5.5 g g^{-1} fibre), wheat bran (6.6 g g^{-1} fibre), apple DF (6.3 g g^{-1}fibre) and some fibre rich supplements (Grigelmo-Miguel & Martõân-Belloso, 1999). The SC of

Table 6
The SC, WHC, and OHC of *G. changii* at various temperatures.

Parameters	RT[A]	37 °C	60 °C	80 °C
SC (mL g^{-1} DW)	5.00 ± 0.28^{d}	7.68 ± 0.13^{c}	9.01 ± 0.06^{b}	10.91 ± 0.06^{a}
WHC (g g^{-1} DW)	6.15 ± 0.06^{d}	9.93 ± 0.08^{c}	11.59 ± 0.04^{b}	13.30 ± 0.08^{a}
OHC (g g^{-1} DW)	3.11 ± 0.60^{a}	1.17 ± 0.22^{c}	1.42 ± 0.26^{d}	1.42 ± 0.13^{b}

Values are Mean ± SEM, n = 3.
(a–d) mean with different superscripts within the same row are significantly difference ($p < 0.05$, ANOVA, Duncan).
[A] RT: Room temperature. Dry weight (DW); Swelling capacity (SW); Water holding capacity (WHC); Oil holding capacity (OHC).

G. changii was significantly increased by 118.20% with the increase in incubation temperature. The differences in SC and WHC among the seaweed samples might be attributed to the different protein conformations and the variations in the number and nature of the water binding sites on the protein molecules (Wong & Cheung, 2000).

For the application in food industry, the high values of WHC of DFs indicate that this material could be used as a functional ingredient for adjustments in texture of meat products and salad dressing for producing low calorie foods, whereas with low values, it is also important when interest lies upon sugar substitutes in low calorie food products such as extruded snacks, corn flakes, cookies, and crackers (Carvalho et al., 2009; Grigelmo-Miguel & Martõân-Belloso, 1999).

OHC is another functional property of food ingredients used in formulated food for consumption. Ingredients with a high OHC values allow the stabilisation of food emulsions and high fat food products. From the current study, the OHC of *G. changii* at 24 °C was significantly higher than OHC at 40 °C, 60 °C and 80 °C. The OHC of *G. changii* was comparable to that of *G. fisheri* (1.79 g g^{-1} DW) (Benjama & Masniyom, 2012). The value was also higher than other seaweeds previously reported (Wong & Cheung, 2000).

4. Conclusion

In conclusion, this study discovered that *G. changii* contained high levels of ash and DFs mainly SDF and relatively high AAs and FAs, which can contribute positively to human nutritional requirements and consumptions. The nutritional compositions together with their physicochemical properties found in *G. changii* can become an important functional ingredient in the food industry. Presently, *G. changii* is found in small scale farming only in tropical water. The studies showed high end potentials that will definitely encourage more commercial large scale farming. With the government's grants and encouragement in seaweed farming, it is envisaged that economic impact on large scale production of *G. changii* will be able to help exports worldwide.

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