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

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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

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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