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Research Article

Proximate, Mineral and Amino acid Composition of Dried Laver (*Porphyra* spp.) Seaweed

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Abstract

Major nutritional compositions of dried laver (*Porphyra* spp.) were evaluated in this study. Findings showed that dried laver contains: moisture (9.34-9.46%), crude protein (42.939–43.041%), dietary fibres (31.598-31.662%), total ash (10.267-10.333%), and crude fat (0.45-0.53%), and total carbohydrate (36.64-37.00%). The total energy value was estimated to be 8,467.69 $KJ.kg^{-1}$. Among the mineral elements (Ca, Mg, K, Na, Cu, Zn, Fe and Mn) analyzed, potassium was the most abundant (1390.76 -1399.24 mg/100 g), while copper was present in the lowest concentration (1.36-1.40 mg/100 g DW). The total and free essential amino acids were 14412.69 mg/100 g and 56.06 mg/100 g respectively. Glutamic, alanine and aspartic amino acids were high in the total amino acids, while, arginine was high in the free amino acids. The dried laver showed appreciable nutritional composition and energy value thus, laver could be an alternative ingredient in the food products.

Keywords: Edible seaweed, dried laver, proximate analysis, amino acids, mineral element, energy value.

Introduction

Aquaculture is the fastest growing food production sector that can play a role in solving the challenges of increasing global food demand (FAO, 2014). In the year 2014, total aquaculture production in the world was increasing to more than 100 million tonnes. Among this, 27 million tonnes were aquatic plants, which are commonly seaweeds that contributed 5.6 billion US$ in the world's economy (Stévant *et al.,* 2017). Asian countries are the most seaweed producers and consumers since dates back to the ancient times. China is one of the leading global producers, which is more than 13 million tonnes per year (Admassu *et al.,* 2015; Cian *et al.,* 2015; Stévant *et al.,* 2017). At present, more than 10,000 different species of seaweeds are well-known and classified as red (Rhodophyta), brown (Phaeophyta) or green (Chlorophyta) seaweeds based on their pigmentation (Benjama and Masniyom, 2011; Collins *et al.*, 2016).

Laver (*Porphyra* spp.) is a red seaweed, popular side dishes of Asian countries where rice is the stable food (Hwang and Thi, 2014). Traditionally, laver is known as Kim in Korea, nori in Japan, and zicai in China (Rao *et al.*, 2007; Hwang *et al.*, 2013). Laver contains various nutritional compositions: high in essential amino acids, essential fatty acids, minerals and vitamins, and bioactive compounds and therefore, it is recognized as a health food (Thi and Hwang, 2014). Lavers are mostly consumed in dried form to improve palatability.

Most previous studies have focused on fresh seaweeds. Some studies have reported that thermal treatments can alter the chemical composition or their chemical forms. Studies on chemical components such as proximate composition, amino acids and mineral contents have a direct role on the characteristics and nutritional quality of food products (Wong and Cheung, 2000; Kaur and Singh, 2005; Kumar *et al.*, 2014; Yaich *et al.*, 2011). As far as the authors knowledge, limited literature are available on nutritional composition of commercial dried laver (*Porphyra* spp.) cultivated in China. Therefore, the objective of the present study was to evaluate major nutritional composition (macro and micro nutrients) of dried laver cultivated in China.

Materials and methods

Materials: The commercial dried laver (*Porphyra* spp.) was obtained from Rudong Laver farming and processing Industry (Nantong, Jiangsu, China). It was ground in an IKA grinder model A11BS25 (IKA werk, Staufen, Germany). The powder was then stored in airtight plastic bags at temperature (-20°C) prior to analysis. All chemical reagents used in the analysis were of analytical grade.

Proximate analysis: Total nitrogen content was determined using a standard micro-Kjeldahl method (Ragab *et al.*, 2004) and converted to total protein (N% X 6.25).

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Residual moisture, total fibre, lipids and total ash contents were determined using the official methods (AOAC, 1995). Total carbohydrates (*TC*) were calculated by difference as follows:

$$TC = 100 - (m_P + m_L + m_W + m_A) \qquad (1)$$

Where *m*P, mL, *m*W and *m*A is content of crude protein, crude lipids, water and ash, respectively.

Energy: Food energy value of dried laver was determined using the Atwater conversion factor as:

$$\text{Energy} = [37\ \text{kJ.g}^{-1} \times \text{mL} + 17\ \text{kJ.g}^{-1} \times \text{mP} + 17\ \text{kJ.g}^{-1} \times \text{mC}] \quad (2)$$

Where, *m*P, *m*L, and *m*C is content of crude lipids, crude protein and available carbohydrate, respectively (expressed in kilojoule per kilogram of food).

Determination of mineral contents: Total Na, K, Mg, Ca, Zn, Fe, Cu, and Mn concentration in dried laver sample were determined using a modified method (Jung *et al*., 2015) . Dried laver sample (1 g) was weighed in porcelain crucible and dry-ashed in muffle furnace at 550°C. The ash was dissolved in 5 mL of HNO3/HCl/H_2O_2 (1:2:3) and heated gently on a hot plate until brown fumes disappeared. Double distilled water (5 mL) was added into the acid digest and heated until colorless. The solution in each crucible was transferred in to a 50 mL volumetric flask and filtered through whatman No. 42 filter paper. Finally, the volume was made up-to mark with deionized water and the minerals were analyzed by Atomic Absorption Spectroscopy (Varian Spectra AA-220 FS, Varian Inc., Australia). The values were expressed in mg/100 g of the sample on dry basis.

Amino acid analysis: Amino acids (total and free) were determined using acid digestion following the procedure of Cian *et al*. (2014) with slight modification. Samples (100 mg) in triplicate were hydrolyzed with 8 mL of 6 mol·L^{-1} HCl under nitrogen and incubated in an oven at 120°C for 22 h. Hydrolyzed samples were injected in to analytical HPLC column of 4.6 × 250 mm I.D, 5 µm particle size (Agilent Technologies, Palo Alto, California, USA) system with ophthaldialdehyde (OPA) precolumn derivation. The determination was made by reverse-phase HPLC (RP-HPLC) (HP-Agilent 1100 model, Agilent Technologies) assembly system at 338 nm detection, 1.0 mL·min^{-1} flow rate and 40°C column temperature. Mobile phase A was (7.35 mmol·L^{-1}) sodium acetate/trimethylamine/tetrahydrofuran (500: 0.12: 2.5, v/v/v), adjusted to pH 7.2 with acetic acid, while mobile phase B (pH 7.2) was (7.35 mmol·L^{-1}) sodium acetate/methanol/acetonitrile (1: 2: 2, v: v: v). The amino acid composition was expressed as milligram per 100 gram of samples.

Statistical analysis: The data were calculated using SPSS software package (Version 17.0) (SPSS, Chicago, Illinois, USA). All experiments were conducted in triplicate and the values represent the average mean of three independent determination and standard deviation.

Results and discussion

Proximate compositions: The proximate compositions of dried laver are shown in Table 1. The moisture content of dried laver (9.34-9.46%) recorded in this study was lower than the reported for *P. columbina* (12.80%) (Cian *et al*., 2014). The lower moisture content could be attributed to the water lost during drying of laver. This lower moisture content can be an indication of quality indices for effective storage of commercial dried laver with promising shelf stability for long term utilization. The crude protein content of dried laver was 42.99±0.051 g/100 g DW). Partially agrees with the reported for red and green seaweed (10~47% DW) (Fleurence, 1999), but higher than the reported for *Palmaria palmata* (35% DW) by the same author and for red seaweed laver *Pyropia dentata* (37.7±1.7%) cultivated in Korea as reported earlier (Jung *et al*., 2015). However, the protein content of laver in this study was lower than that of *Porphyra tenera* (47% DW) described by Fleurence, (1999). The difference could be ascribed due to species, harvesting season and location (Fleurence, 2004). In general, red seaweeds have protein content comparable with plant food known for their remarkably high protein content like soybean (Cian *et al*., 2014). The high level of protein in the Laver under study is a plus for the high potential of laver to be used in the formulation of value-added protein-rich food products that can be used in protein supplemention to overcome the proten defficiency.

The ash content (10.30±0.033 g/100 g DW) of dried laver in this study was the highest of all in the reported for *P. columbina* (6.49±0.09 g/100 g), dried laver *P. tenera* (9.07 ±0.29 g/100 g), *P. haitanensis* (8.78±0.12 g/100 g) (Hwang *et al*., 2013), and dried laver *Porphyra yezoensis* (8.01~8.95%) (Kim *et al*., 2014). On the other hand, the ash content of dried laver was considerably lower than the result previously reported for some subtropical red and green seaweeds (21.30–22.8% DW) (Wong and Cheung, 2000), in four Brazilian red seaweeds in the range of 22.5±0.3 to 38.4±0.1 g/100 g (Gressler *et al*., 2010) and *Porphyra tenera* (Nori) (20.59±0.16 g/100 g DW) (Ruperez, 2002). This reduction in ash content might be due to the drying process, species, season of harvest and location (Fleurence, 2004). The crude lipid content of Laver (0.49±0.04 g/100 g DW) was lower than the reported for *Porphyra* species from China (1.0±0.2), and Japan and Korea (2.8±1.0 g/100 g DW) (Dawczynski *et al*., 2007).

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Table 1. Proximate composition and total energy value of commercial dried laver.

Composition	Unit: g/100 g DM
Moisture	9.40±0.056
Crude protein	42.99±0.051
Crude fat	0.49±0.040
Ash	10.30±0.033
Total dietary fibre	31.63±0.032
Total Carbohydrate	36.82±0.180
Available carbohydrate	5.190±0.212
	Energy (Unit: $KJ.Kg^{-1}$)
Carbohydrate	978.69±36.04
Protein	7308.30±8.67
Lipid	181.30±6.80
Total Energy	8,467.69±51.54

Table 2. Mineral content of commercial dried laver (*Porphyra* spp.) (mg/100 g DW).

Macro minerals	Contents (mg/100g)	Micro minerals	Contents (mg/100g)
Na	348.75±1.06*	Fe	12.28±0.32
K	1395.00±4.24	Zn	2.79±0.1
Ca	525.00±1.41	Mn	2.26±0.04
Mg	261.75±1.06	Cu	1.38±0.02

*Values are mean ± SD (n=3).

Generally, seaweeds are reported to be a poor sources of lipids (<4.00 g/100 g DW) (Ortiz *et al*., 2006). The low lipid content is of a great advantage, especially in regards to producing healthy food products that may help to improve the health status of obese people with hypocholesterolemic cases. The total dietary fibre of dried laver was 31.63±0.032 g/100 g DW (Table 1). The total dietary fibre content of dried laver is within the range reported for many algal species by different authors (Escrig and Muniz, 2000; Gómez-Ordóñez *et al.,* 2010). Seaweeds contain quite large amount of dietary fibres (25-75 g/100 g DW) of which the majority is soluble fractions (50-85 g/100 g DW) (Gómez-Ordóñez *et al*., 2010), and the high dietary fibre content of seaweeds makes it nutritionally very important (Escrig and Muniz, 2000; Wong and Cheung, 2000). The total and available carbohydrate content of the laver was 36.82±0.18 and 5.19±0.212 g/100 g DW respectively. Thus, the level of dietary fibres obtained in this study is in accordance with the previously reported level of carbohydrates, indicating that fibres make up the majority of the carbohydrate part of the commercial dried Laver. This could be considered as a useful nutritional characteristic of Laver to use it as an ingredient of functional food that improves bowel function and provide fecal bulk. The energy value of dried laver sample (8467.69±51.54 $kJ.kg^{-1}$) was quite larger which might have been contributed by the high crude protein content (Table 1). This indicates that dried Laver could be used as a great source of daily energy requirement in diet, especially among resource-poor rural households.

Mineral element compositions: The mineral element compositions of the dried laver are given in Table 2. The result showed that the mineral content was: Ca (523.59-526.41 mg/100 g), Mg (260.69-262.81 mg/100 g), K (1390.76 - 1399.24 mg/100 g), Na (347.69-349.81 mg/100 g), and trace minerals with nutritional interest such as: Fe (11.96-12.60 mg/100 g), Zn (2.69-2.89 mg/100 g), Cu (1.36-1.40 mg/100 g), and Mn (2.22–2.30 mg/100 g). The most abundant mineral element was potassium (K) (1390.76-1399.24 mg/100 g), which is recognized as a healthy mineral, while copper (Cu) was present in lowest concentration (1.36-1.40 mg/100 g DW). However, total potassium content of the dried laver in this study was lower than the previously reported value for laver *P. tenera* (2801.286-2802.714) and *P. haitanensis* (2733.255 – 2734.745 mg/100 g DW) from Korea and china, respectively and *P. yezoensis* (1555.00-1862.00 mg/100 g DW) in Korea harvested from four different regional locations (Kim *et al*., 2014). Calcium (525.00 mg/100 g) contents were higher than the previously reported values for *P. tenera* (Ca, 154.4 mg/100 g), *P. haitanensis (Ca,* 460.6 mg/100 g) and *P. yezoensis* (Ca, 252.28-417.00 and Cu, 0.52-0.88 mg/100 g) (Hwang *et al*., 2013; Kim *et al*., 2014). On the other hand, the sodium and magnesium contents were lower than the results reported previously for *P. tenera* (Na, 781, Mg, 420.3) mg/100 g, *P. haitanensis* (Mg, 612.00 mg/100 g) and *P. yezeonsis* (Na, 444-593, Mg, 330-409 mg/100 g) (Hwang *et al*., 2013; Kim *et al*., 2014).

Table 3. Total and free amino acid contents in commercial dried lavers (Units: mg/100g protein).

Amino acids	Total amino acid	Free amino acid	FAO/WHO/HNU pattern[a]	Whole egg protein[b]
Phenyl alanine	1561.00 ± 4.58[(1)]	1.11 ± 0.01[(1)]	1900.0	5700.0
Threonine	1772.62 ± 2.52	1.52 ± 0.02	900.0	5100.0
Methionine	633.00 ± 2.65	4.66 ± 0.06	2500.0	3400.0
Isoleucine	1616.57 ± 0.58	0.39 ± 0.11	1300.0	6300.0
Arginine	2099.00 ± 3.00	41.19 ± 0.21	460.0	6100.0
Leucine	2643.01 ± 3.00	1.11 ± 0.15	1900.0	8800.0
Lysine	1682.49 ± 2.31	1.22 ± 0.34	1600.0	6960.0
valine	2405.00 ± 2.65	4.86 ± 0.31	1800.0	6900.0
Tryptophan	**nd	**nd	500.0	1700.0
EAA[(2)]	14412.69 ± 13.68	56.06 ± 0.55	12,860.0	50,960.0
Aspartic acid	4213.33 ± 1.53	19.32 ± 0.02	880.0	9600.0
Glutamic acid	5527.31 ± 2.08	11.31 ± 0.03	1750.0	12702.0
Alanine	4539.27 ± 2.52	11.12 ± 0.61	260.0	5900.0
Glycine	2351.41 ± 0.58	2.55 ± 0.07	200.0	3300.0
Proline	1434.71 ± 1.53	0.13 ± 0.02	610.0	4200.0
Serine	1202.31 ± 2.00	0.13 ± 0.02	530.0	7600.0
Tyrosine	781.63 ± 1.53	4.87 ± 0.33	460.0	4200.0
*Histidine	485.58 ± 2.52	1.24 ± 0.09	1600.0	2426.0
Cysteine	28.43 ± 2.08	0.45 ± 0.01	1700.0	2426.0
Total AA[(3)]	34976.67 ± 37.63	107.2 ± 2.40	20,850.0	103,314.0

**Histidine: Essential amino acid for children, **nd: not determined,* [(1)] *Values are mean ± SD (n=3),* [(2)] *EAA: Essential amino acid,* [(3)] *Total AA: Total amino acid;* [a]*Amino acids requirement pattern for adults* (WHO/FAO/UNU, 1985), [b]*Whole egg protein* (FAO, 1970).

Many factors including the analytical and processing methods employed, geographical location of harvest, seaweed species, oceanic residence time, season, annual environment, and so forth could affect the mineral contents of seaweed and cause variation(Rao *et al*., 2007; Hwang *et al*., 2013). In a nutshell, the results of this study shows that laver is a good source of dietary mineral elements. Thus, laver could be considered as a viable strategy for counteracting mineral defficiencies among the vulenerable popluation groups, where laver harvesting is large and laver-based foods are highly consumed. Evaluation of mineral contents in any edible seaweed is important to address their potential contribution to human nutrition and their adverse toxic effects (Rao *et al*., 2007). The low content of sodium compared to potassium in the dried Laver implies Na/K ratio of dried laver to be less than 1, suggesting suitability of Laver as healthy food for people that have high risk of blood pressure. Foods with Na: K ratio (a suitable index of body electrolyte balance) of less than one is suggested for prevention of high blood pressure. Unlike potassium, the high content of sodium in food risk factor cardiovascular and other non-communicable diseases (Campbell *et al*., 2011).

Amino acid composition: The amino acid (total and free) composition of dried laver (*Porphyra* spp.) is presented in Table 3. A total of seventeen (17) different amino acids were determined in this study. Except Tryptophan (which was not determined), all essential amino acids were found in dried laver in different quantities.

The dried Laver had remarkably a high level of glutamic acid (5527.33±2.08 mg/100 g), alanine (4539.33±2.52 mg/100 g) and aspartic acid (4213.33±1.53 mg/100 g) in its total amino acids, which are responsible for the special flavor and taste of laver. Similar observation was previously reported in red and green seaweed from Hong Kong (Wong and Cheung, 2000), and in four Brazilian red algae species (Gressler *et al*., 2010). However, arginine was present in higher concentration in its free amino acids (41.19±0.21), followed by aspartic acid (19.32±0.020 mg/100 g), glutamic acid (11.31±0.08 mg/100 g) and alanine (11.12±0.61 mg/100 g). Cysteine was present in the lowest concentration in total amino acid whereas proline and serine were lowest in free amino acids. The total and free amino acid content of essential amino acids was 14412.69±13.68 and 56.06±0.55 respectively. From the obtained results, it appeared that dried laver had reasonable concentrations of essential amino acids (except tryptophan) that are very important to human nutrition. These are methionine, leucine, isoleucine, lysine, phenylalanine, arginine, threonine and valine. The levels of different essential amino acids ranged from 633.00 to 2643.00 mg/100 g protein DW. Dried laver was rich in arginine (2099.00±3.00), leucine (2643.01±3.00), valine (2405.00±2.65) and threonine (1772.62±2.52) for total amino acids (Table 3). This result is in agreement with previously reported for two red seaweed species (*Gracillaria fisheri and G. tenuistipitata*) (Benjama and Masniyom, 2012). The non-EAA, namely histidine, aspartic acid, glutamic acid, serine, proline, glycine, alanine, tyrosine, and cysteine were analyzed.

Fleurence (1999) reported that a large part of amino acid fractions for most seaweeds are constituted by aspartic and glutamic acids. This, same author has also reported, the level of these amino acids might be lower in red seaweed species than the other species, example, *Palmaria palmate* (14,000 mg/100 g) and *P. tenera* (19,000 mg/100 g) of the total amino acid. The sum of aspartic (5527.33 mg/100 g) and glutamic acid (4213.33 mg/100 g) in this study was 9740.66 mg/100 g of proteins for the total amino acid. In the free amino acid fraction, the sum total of aspartic (19.32 mg/100 g) and glutamic acid (11.31 mg/100 g) was estimated to be 30.63 mg/100 g. The EAA was found to be 14412.69 mg/100 g and 56.06 mg/100 g for total and free amino acids with a ratio of 0.74: 1 and 0.52: 1 (EAA: non-EAA), respectively. This result was favorably compared with the value reported for *Porphyra* species from Korea and Japan, however, lower than *Porphyra* species in china which was determined by Wong and Cheung (2000). Dried laver contained small concentrations of free amino acids. This indicates that most amino acids and small peptides are encrypted with the original protein and needs *in vivo* or *in vitro* digestion to release them so that can perform their specific biofunctionality in the body. In general, dried laver had significantly higher concentration of both essential and non-essential amino acid content, which compared and found to be greater than FAO/WHO recommended pattern.

Conclusion

In conclusion, the commercial dried laver analyzed in this study has a great potential as food product or as an ingredient in other food products due to its quite unique nutrient composition with high protein contents, moderate ash and dietary fibre contents. Dried laver (*Porphyra* spp.) showed higher gross energy value and thus could be used as potential source of dietary calorie. Commercial dried laver was furthermore found to have mineral contents including important trace elements Zn and Fe which may provide adequate quantity of minerals to meet the mineral requirements of human body. Dried laver also contains various essential and non-essential amino acids important in body synthesis. With further investigation of more nutritional quality parameters and functional components, dried laver could be used in different food applications.

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