

Proximate Composition, Amino Acid, Mineral, and Heavy Metal Content of Dried Laver

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ABSTRACT: Laver, a red algae belonging to the genus *Porphyra*, is one of the most widely consumed edible seaweeds. The most popular commercial dried laver species, *P. tenera* and *P. haitanensis*, were collected from Korea and China, respectively, and evaluated for proximate composition, amino acids, minerals, trace heavy metals, and color. The moisture and ash contents of *P. tenera* and *P. haitanensis* ranged from 3.66~6.74% and 8.78~9.07%, respectively; crude lipid and protein contents were 1.96~2.25% and 32.16~36.88%, respectively. Dried lavers were found to be a good source of amino acids, such as asparagine, isoleucine, leucine, and taurine, and γ -aminobutyric acid. K, Ca, Mg, Na, P, I, Fe, and Se minerals were selected for analysis. A clear regional variation existed in the amino acid, mineral, and trace metal contents of lavers. Regular consumption of lavers may have health benefits because they are relatively low in fat and high in protein, and contain functional amino acids and minerals.

Keywords: dried laver, proximate composition, amino acids, minerals, heavy metals

INTRODUCTION

Laver (*Porphyra* spp.) has long been cultivated in Asian countries, mainly Korea, Japan, and China, which are the world's major areas of laver production (1). The genus *Porphyra*, traditionally known as *kim* in Korea, *nori* in Japan, and *zicai* in China, has been subject to increased demand due to the increasing popularity of oriental cuisine and macrobiotic diets in Western countries in recent years (2,3). Lavers are rich in essential amino acids such as methionine, threonine, and tryptophan, and contain an abundance of minerals such as potassium (K), phosphorus (P), magnesium (Mg), sodium (Na), and calcium (Ca) (2). From a nutritional perspective, lavers are characterized by high concentrations of fiber and minerals (4,5), are low in fat, and have relatively high levels of protein (6,7). Seaweed consumption has increased in Western countries in recent years because of its nutritive value and health benefits (8). Seaweeds provide a bioavailable, alternative dietary source of macro, trace, and ultratrace elements (5,9). Therefore, the value of seaweed as a new health and functional ingredient is attracting much attention (10).

In contrast to land plants, the chemical composition of

seaweeds, including lavers, has been poorly investigated. Seaweeds live in a harsh environment constantly exposed to various environmental stresses (10). The chemical and nutrient compositions of seaweeds are determined by species and environmental conditions such as habitat, light, water temperature, and salinity (11,12). Seaweeds can accumulate essential mineral elements, such as Ca, Mg, cobalt (Co), selenium (Se), iron (Fe), and iodine (I), from seawater at higher rates than land vegetables (13). However, as a result of environmental pollution, seaweeds can also concentrate several toxic elements, such as arsenic (As), lead (Pb), and cadmium (Cd) (14,15).

Previous studies have been performed on raw seaweeds; however, lavers are mostly consumed in the dried form to improve palatability. Despite the fact that most laver is manufactured and consumed in dried form, few studies have conducted chemical analyses of commercialized dried laver. Some studies have reported that thermal treatments can alter the mineral and heavy metal content (or their chemical forms), leading to a more toxic produce than the original fresh state (16). However, Sartal et al. (16) reported that 34~71% of as in four different seaweeds (Kombu, Wakame, Nori, and sea lettuce) were removed during cooking and released into

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the cooking water.

In the present study, we investigated the proximal levels of amino acids, minerals, and heavy metals in dried laver obtained from Korea and China. The results emphasize the differences between species and regional growing conditions, and enhance our nutritional knowledge of laver.

MATERIALS AND METHODS

Chemicals and materials

Lavers, purchased from a local market in Wando, Korea and Jiangsu, China on December, 2012, were collected and dried. Samples were blended to obtain homogeneous mixtures and stored in airtight plastic bags (due to their hygroscopic nature) until undergoing analytical treatment. Organic solvents were purchased from Burdick & Jackson (Batavia, IL, USA). Ninhydrin reagent and a 45 amino acid standard mixture were purchased from Pickering (Pickering Laboratories, Inc., Mountain View, CA, USA). All reagents and chemicals used were of analytical grade.

Proximate analysis

Residual moisture content was determined by drying to a constant weight at 105°C in an oven (EYELA, Tokyo Rikakikai Co., Tokyo, Japan). Ash content was determined using a previously published method (17). Briefly, laver samples were incinerated in a digitally controlled Hobersal HD-230 furnace (Kukje Engineering, Daejeon, Korea). Temperature was gradually increased to 550°C and then maintained for 16 h. Ash mass was quantified gravimetrically. Crude lipids were extracted from the laver powder in a Soxhlet extractor (Soxtec System HT6, Tecator AB, Hoganas, Sweden) using ethylether. The crude lipid content was determined gravimetrically following oven-drying of the extract at 105°C overnight. Nitrogen content was determined using the micro-Kjeldahl method (17). The crude protein content was calculated by multiplying the Kjeldahl nitrogen by a factor of 6.25. About 0.1 g pulverized sample was taken for protein analysis. All determinations were performed in triplicate, and the data are expressed in terms of mean±standard deviation (SD).

Color analysis

Laver color was determined with a colorimeter (Model CR-400, Konica Minolta Business Technologies Inc., Tokyo, Japan) using a 1.4 cm measuring aperture and a white background. Before the test, the instrument was calibrated using standard black and standard white glass provided by the manufacturer. The L^* , a^* , and b^* com-

ponents of the CIELAB space were recorded, where L^* indicates lightness, a^* indicates chromaticity on a green (−) to red (+) axis, and b^* indicates chromaticity on a blue (−) to yellow (+) axis.

Amino acid analysis

Free amino acids were analyzed using an Agilent 1100 system (Agilent Technologies, Santa Clara, CA, USA). Separations were performed with a cation exchange column (3×250 mm, 8 μm particle size; Pickering Laboratories Inc., Mountain View, CA, USA) at 40°C with a flow rate of 0.3 mL/min. The reactor was a Pinnacle PCX (Pickering Laboratories Inc.) and the temperature was 130°C. The laver was cut into small pieces weighing ~10 g, and was mixed with 150 mL 70% ethanol and extracted for 2 h at 80°C. The mixture was centrifuged at 5,000×g for 20 min, and the upper layer was saved to another tube. The extraction was performed three times. Solvent fractions were combined and evaporated to dryness in a vacuum at 45°C. The residue was redissolved in a 50 mL mass flask with lithium. The mixture was centrifuged and filtered through a 0.2 μm syringe filter. The mixture was diluted 40-fold using 10 μL column injections of lithium diluents (pH 2.36). The amino acid concentrations of lavers were calculated from calibration curves based on amino acid standard mixtures (Pickering Laboratories Inc.).

Mineral and heavy metal analysis

Approximately 0.5000 g pulverized laver was placed in a beaker with 1 mL HNO₃. The mixture was reacted at 50°C on a hot plate to allow the sample to be digested by HNO₃ in the fume hood. After acid digestion, the beaker was carefully removed from the hot plate and the contents were left to cool for 30 min, also allowing the acid to evaporate. After evaporation of the acid, the digested samples were transferred to a 50 mL volumetric flask with deionized water (1~5% acid concentration). Ca, Fe, K, Mg, Na, and P were analyzed by inductive coupled plasma-atomic emission spectroscopy (ICP-AES, Jobin Yvon, Longjumeau, France). Other minerals (I, Se) and heavy metal ions were analyzed by inductively coupled plasma mass spectrometry (ICP-MS, Agilent Technologies). Triplicate determinations for each element were carried out. The concentration of elements was determined from calibration curves of the standard elements.

Statistical analysis

Experimental values were mean±SD from three separate experiments. Significance was assessed using ANOVA-tests in SPSS 17.0 (Statistical Package for the Social Sciences, SPSS Inc., Chicago, IL, USA). A probability value of $P<0.05$ was considered significant.

RESULTS AND DISCUSSION

Proximate composition

Table 1 shows the proximate composition of laver. The moisture content of *P. tenera* and *P. haitanensis* were 3.66% and 6.74%, respectively. The *P. haitanensis* contained significantly higher ($P < 0.01$) moisture content than in *P. tenera*. Some red seaweeds (*Hypnea japonica* and *H. japonica*) have been found to have higher moisture contents than our values, at 9.95~10.9% (12).

The ash content of the *P. tenera* and *P. haitanensis* was similar across samples at 8.78~9.07%, with no statistically significant differences between species and growing region. The ash contents of lavers in our study were lower than those reported for alternative seaweed species. More specifically, *Ulva lactuca* and *U. pertusa* were found to contain 24.6% and 24.7% ash by dry weight (DW), respectively (18,19).

P. tenera contained higher crude lipid content (2.25%) than *P. haitanensis*. Our results are similar to those reported by Fleurence et al. (20), and the 3.4% DW for *Porphyra umbilicalis*, 1.6% DW for *P. palmate*, and 1.4~1.5% for red seaweeds (*Hypnea japonica* and *H. japonica*) reported by Wong et al. (12). However, Yaich et al. (21) reported the lipid content of *Ulva lactuca* seaweed collected in Tunisia to be 7.8%, which is higher than our results.

The crude protein content of the two different species were between 32.16% and 36.88%, within the range for red seaweed (10~47%) reported by Fleurence (22). The variation in the protein content of laver may be due to the different species and processing methods (22). Wong and Cheung (12) reported that the crude protein content was 21.3~22.8% DW in two subtropical red seaweeds (*Hypnea charoides* and *Hypnea japonica*) and one green seaweed (*Ulva lactuca*). Norziah and Ching (23) reported that *Porphyra* spp. contained high levels of protein, comparable to those of high-protein plant-based foods such as white soybean (33.8% protein).

Denis et al. (24) reported that the composition of *Grateloupia turuturu*, edible red seaweed in France, was 18.5% ash, 22.9% total protein, and 2.6% total lipid. Red seaweed, especially laver (*Porphyra tenera*), pos-

Table 1. Concentration of moisture, ash, crude lipid, and crude protein in laver (%)

	<i>P. tenera</i>	<i>P. haitanensis</i>
Moisture	3.66±0.25	6.74±0.51*
Ash	9.07±0.29	8.78±0.12
Crude lipid	2.25±0.29*	1.96±0.4
Crude protein	36.88±0.90	32.16±1.21

Data are mean±SD from three separate experiments. The values marked with an asterisk indicate significant differences with other treatment ($P < 0.05$).

sesses a high level of protein, as much as 47.5% (25). Differences in proximate composition may be attributed to factors such as climate, temperature, pH, geographical differences, species, and season (22,26).

Color analysis

Table 2 shows the color parameters of the different species of lavers. *P. tenera* had higher lightness (L^*) values but not significantly different compared to *P. haitanensis* ($P > 0.05$). *P. tenera* had lower redness (a^*) than *P. haitanensis*. No previously reported color analysis results for laver exist and therefore no data with which to compare our data. The color differences we found may be characteristics of laver, or be representative of their chemical composition.

Amino acid analysis

The quantitative measurement of amino acids was conducted using an Agilent 1100 system. The amino acid composition of laver is presented in Table 3. *P. tenera* and *P. haitanensis* were good sources of amino acids such as taurine, alanine, and glutamic acid. *P. tenera* contained 13 different amino acids, and was particularly rich in asparagine, isoleucine, leucine, and GABA. *P. haitanensis* contained high amounts of threonine, serine, asparagine, and alanine. Both *P. tenera* and *P. haitanensis* contained 141.98 and 171.37 mg of aspartic acid in 100 g DW, respectively. The high levels of these amino acids are responsible for the special flavor of the seaweed (27). All lavers also contained alanine (936.28~1218.71 mg/100 g

Table 2. Color parameters of lavers

Sample	<i>P. tenera</i>	<i>P. haitanensis</i>
Lightness, L^*	40.10±0.75	37.02±1.38
Redness, a^*	0.36±0.07	0.44±0.11
Yellowness, b^*	1.66±0.11	1.47±0.12

Data are mean±SD of four separate experiments.

Table 3. Concentration (mg/100 g) of amino acids in laver

	<i>P. tenera</i>	<i>P. haitanensis</i>
Taurine	979.04±37.41*	646.55±12.51
Aspartic acid	141.98±2.63	171.37±1.02
Threonine	31.80±1.02	86.43±1.36*
Serine	20.02±2.56	44.81±1.87*
Asparagine	22.37±0.25	86.55±2.54*
Glutamic acid	843.35±34.55*	277.45±10.54
Glycine	22.06±0.38	26.11±1.81
Alanine	936.28±12.33	1,218.71±25.64*
Citrulline	77.80±4.58	71.32±3.25
Valine	33.48±7.55	—
Isoleucine	46.67±2.08	49.88±1.97
Leucine	27.92±2.30	33.22±1.65
γ -aminobutyric acid	31.34±1.25	—

Data are mean±SD of four separate experiments. The values marked with an asterisk indicate significant differences with other treatment ($P > 0.05$).

DW) and glycine (22.06~26.11 mg/100 g DW). Seaweeds containing these compounds have a sweet flavor (28).

Taurine was the most abundant amino acid in red algae, especially *Porphyra* species. The *P. tenera* and *P. haitanensis* contained high levels of taurine, 975.04 mg and 645.55 mg in 100 g DW, respectively. Dawczynski et al. (8) detected significantly high levels of taurine in *Porphyra* sp. from Korea and Japan amounting to 4 g/16 g nitrogen compared to *Porphyra* sp. collected from China (2.4 g/16 g nitrogen) or brown algae varieties (0.1~0.6 g/16 g nitrogen). Taurine is a free amino acid that is found in most tissues, with particularly high levels in the heart, blood, and developing brain (29). Red seaweeds, especially lavers (*Porphyra* sp.) are good sources of taurine, which is a main ingredient of bile and aids in the digestion of fats and the absorption of vitamins that are fat-soluble (29). Increased dietary intake of taurine may have beneficial effects on the heart and may help battle diabetes and hypertension (30-32).

GABA is a non-protein amino acid that is widely distributed in nature and well-known for its physiological functions, such as the induction of hypotension and diuretic effects, and the inhibition of neurotransmitters in the central nervous system (33,34). The amino acid profiles of red and green seaweeds are clearly different. Red seaweeds have been found to have higher levels of sulfur-containing amino acids (16.2~17.3 g/100 g DW) than green seaweeds (6.30 g/100 g DW) (12).

Mineral analysis

Table 4 shows the concentration of minerals in *P. tenera* and *P. haitanensis*. Eight elements, K, Ca, Mg, Na, P, I, Fe, and Se, were found in both species of lavers. K was the most abundant (27,340~28,020 µg/g DW) in both *P. tenera* and *P. haitanensis*. *P. tenera* contained high amounts of K (28,020 µg/g DW), Na (7,811 µg/g DW), and I (3,108 µg/g DW) compared to *P. haitanensis*. *P. haitanensis* contained high levels of Ca (4,606 µg/g DW), Fe (700 µg/g DW), and Mg (6,120 µg/g DW) compared to *P. tenera*. Both *P. tenera* and *P. haitanensis* contained relatively high levels of I (2,407~3,108 µg/g DW).

Table 4. Concentration (µg/g) of minerals in laver

	<i>P. tenera</i>	<i>P. haitanensis</i>
Ca	1,514±4.17	4,606±4.33*
Fe	180.0±2.03	700.5±2.37*
K	28,020±7.14	27,340±7.45
Mg	4,203±3.30	6,120±5.49*
Na	7,811±7.20*	1,992±2.10
P	8,201±6.90	8,854±4.09
I	3,108±4.24*	2,407±3.65
Se	204±1.03*	126±0.98

Data are mean±SD of three separate experiments. The values marked with an asterisk indicate significant differences with other treatment ($P<0.05$).

Seaweeds, including laver, are characteristically a good source of I. Dried kombu (*Laminaria japonica*) has the highest I content of all seaweeds, approximately 2,700 mg/kg (35). Regular consumption of laver may decrease the incidence of breast cancer due to its high I content (36). In the present study, Se levels in laver were 126~204 µg/g DW (Table 4). Se is an essential micronutrient for animals and humans, and it plays important biological roles as an antioxidant, a regulator of thyroid hormone metabolism, and as an anti-carcinogenic agent.

Several studies have shown that the alkalinity of seaweed confers numerous health benefits, such as improving thyroid function and lowering the acidity levels in the body, thus preventing the development of degenerative illnesses such as cancer and heart disease (37). Mineral content has been shown to vary according to the seaweed species, oceanic residence time, geographical place of harvest, wave exposure, season, annual environment, type of processing methods, and so forth (13,38). Mineral content in laver is higher than that of land plants and animal products (11,39). Thus edible marine seaweeds may be an important source of minerals because some of these trace elements are either absent from, or only very minor in, land vegetables (1,11,39).

Heavy metal analysis

Table 5 shows the heavy metal concentration in *P. tenera* and *P. haitanensis*. Mercury (Hg) levels in both species of laver were less than 100 ng/g DW, the limit of detection of the methodology. However, a relatively high level of Pb has been detected in *P. haitanensis* with concentrations of 1,566 ng/g DW. The Pb content of *P. tenera* was 256 ng/g DW and was considered a moderate level compared to that of *P. haitanensis*. The Pb contents varied depending on the species of seaweed. Almela et al. (40) reported that the Pb content of red and brown seaweeds were 554 ng/g DW and 598 ng/g DW, respectively. On the other hand, several researchers have detected much higher amounts of Pb (2,200~14,200 ng/g DW) in red and brown seaweeds (39).

The Cd level in *P. haitanensis* (3,408 ng/g DW) was relatively higher than *P. tenera* (1,629 ng/g DW). Almela et al. (40) found a wide range of Cd concentrations (19~3,000 ng/g) in *Porphyra* of different origins including

Table 5. Concentration (ng/g) of heavy metals in laver

	<i>P. tenera</i>	<i>P. haitanensis</i>
Hg	<100	<100
Pb	256±0.12	1,566±0.22*
Cd	1,629±0.30	3,408±0.45*
As	32,027±7.44	43,895±12.04*

Data are mean±SD of three separate experiments. The values marked with an asterisk indicate significant differences with other treatment ($P<0.05$).

those from Korea and Japan. van Netten et al. (41) reported lower Cd levels at 270~830 ng/g for *Porphyra* from Japan.

The level of As in *P. tenera* was 32,027 ng/g DW, and 1.37 times higher in *P. haitanensis* (43,895 ng/g DW). Usually, the concentration of as is higher in marine organisms than in terrestrial ones because seafood can accumulate more As than other foods (42).

Seaweed has a high accumulation capacity for heavy metals and has been used as a bio-indicator of contamination of marine environments (43). Environmental factors such as water salinity, water temperature, and pH may affect metal accumulation (43-45). Ródenas de la Rocha et al. (15) reported that Asian seaweeds had higher levels of Pb (623~1,265 ng/g DW) and Cd (1.6~3.1 ng/g DW) than their European counterparts (Pb: 317~403 ng/g DW, Cd: 0.40~1.70 ng/g DW); this likely reflects different levels of environmental pollution, as the concentrations of heavy metals vary widely between the areas studied. Several countries, such as France, the United States, and Australia, have established specific regulations for toxic elements in edible seaweed; however, most other countries have no such regulations (40). More importantly, the levels of toxic heavy metals must be monitored along with developing human health thresholds.

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AUTHOR DISCLOSURE STATEMENT

The authors declare no conflict of interest.

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