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The Analysis of Proximate Composition, Minerals and Amino Acid Content of Red Alga *Pyropia dentata* by Cultivation Sites[1a]

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ABSTRACT

In this study, the proximate composition of *Pyropia dentata* was compared by the analysis of minerals and amino acids that were produced at Seocheon, Chungcheongnam-do and at Wando and Jangheung, Jeollanam-do. Moisture, ash and crude proteins were analyzed using the AOAC method, and crude lipid was analyzed using the Soxhlet method, and inorganic analysis was performed using ICP-OES. Amino acid was used for the amino acid analyzer. The proximate composition analysis, moisture content, maximum values were displayed in Wando while the maximum content of ash appeared at Jangheung's Pyropia. Crude proteins was appeared at Seocheon's *Pyropia* and the maximum amount of crude lipid, carbohydrate was appeared in Wando's *Pyropia*. Inorganic analysis, calcium, potassium, magnesium, sodium, phosphorus, cobalt, chromium, copper, iron, manganese, nickel, zinc were analyzed from the Seocheon's *Pyropia*, however, cobalt, chromium, and nickel was not detected in Wando's *Pyropia*. For amino acid analysis, a total of 17 amino acids were detected: leucine, valine, aspartic acid, glutamic acid, and the amount of alanine accounted for about 57 % of total amino acids(26.1-28.7 %). The proximate composition, minerals, and amino acid of Pyropia dentata were different depending on the coastal ecological habitats.

KEY WORDS: SEAWEED, NUTRITIVE SALTS, CARBOHYDRATES

INTRODUCTION

Seaweed is a globally popular health food which was recently used as medicine and fertilizer. (Choi and Choi, 2002). Although it is a low calorie food containing only about 1 % fat (Oh *et al*., 2013), it is abundant in vitamins, minerals, dietary fibers and essential amino acids. In particular, carbohydrates and proteins account for approximately 70 % of its entire composition (Park *et al*., 2000). Among edible seaweed species, laver makes up the largest share in the seaweed processed food category (Kim *et al*., 2003). Also, as it became known that sugar and dietary fiber contained in a carbohydrate are related to the prevention of general diseases, seaweeds are receiving a broader spotlight for their outstanding nutritional qualities. Amino acids that are compose of protein are essential nutrients and are related to the body's metabolism (Furst and Stehle, 2004a; Kwak *et al*., 2010b). Minerals are also known for their anti-aging and cancer preventive effects. Laver was reported to be rich in microelement minerals (Kang, 1970a; Lee *et al*., 2012b).

Also, as laver receives worldwide attention these days, the Korean government designated it as one of ten strategic export items and established a fostering plan to dominate

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global markets. It also proposed an international laver standard criteria to the Codex Regional Coordinating Committee for Asia (CCASIA) in 2010, and is striving to maintain and develop export markets (Hwang, 2013).

Up to the present, with regard to laver research, only a partial physicochemical analysis of laver has been conducted. Therefore, this study aimed to analyze and compare the proximate composition, minerals and amino acids of *Pyropia dentata* produced in Seocheon, Chungcheongnam-do; and Wando and Jangheung, Jeollanam-do.

MATERIALS AND METHODS

1. Material

Laver used in the experiment was *P. dentata*, which was produced in February 2014 and purchased in Seocheon, Wando and Jangheung. After transporting the laver to the laboratory, microscopic examination and identification was performed using an optical microscope (× 200) and consulting research documents. The laver was used after drying in a pyrostat (26 ℃) for 24 hours (Lee, 2008).

2. Proximate Composition Analysis

With regards to proximate composition, this study measured moisture, ash, crude proteins, crude lipids and carbohydrate. The 105 ℃-normal pressure drying method; the 550 ℃-ash method; and the semi-micro Kjeldahl method were applied to the moisture, ash and crude proteins, respectively, in accordance with AOAC (1995). In case of carbohydrates, it was indicated by the value after subtracting the content (%) of the moisture, ash, crude proteins and crude lipid from 100 %.

3. Mineral Analysis

Concerning the mineral analysis, this study referred to Hwang(2013)'s method, and made some adjustments to suit this experiment. After placing 0.5 g of sample; 7 ㎖ of nitric acid solution; and 1㎖ of hydrogen peroxide in a teflon container, the contents decomposed after heating them for 30 minutes in a heating block (200 ℃) until they turned into a clear yellow solution. After eliminating the acid by cooling down the decomposed sample at room temperature for 30 minutes, the distilled water was added until the 50 ㎖ mark was reached. This was used as an analysis sample, and an inductively coupled plasma spectrum analyzer (Vista-PRO, Agilent Technologies, GERMANY) was used for the analysis.

4. Amino Acid Analysis

After adding 6 N HCl 20 ㎖ to 1g of sample, nitrogen gas was injected, and they were hydrolyzed for 20 hours in 110 ℃. The hydrolyzed sample was glass filtered (2.5 ㎛), and the acid was removed in 50 ℃ with a rotary evaporator (80 rpm). After adding 0.2 M of sodium citrate buffer (pH 2.2) to the acid-removed sample, which was administered at the constant volume of 10 ㎖, 1 ㎖ was put into a syringe filter(0.2 ㎛) for filtering. After the process, the sample was diluted by 40 times and was analyzed with an amino acid analyzer (L-8900, HITACHI, JAPAN).

5. Statistics Analysis

With regard to the statistics process of the results, this study used ANOVA of the SPSS version 17.0 (Chicago, IL, USA) program to verify the significance between the processed groups, and analyzed them with Duncan's multiple test.

RESULTS AND DISCUSSION

1. Proximate Composition Analysis

The analysis results of proximate composition content are shown in Table 1. With regard to Seocheon laver, its carbohydrate content was 45.7 ± 2.3 %, which was composed of moisture (8.8 ± 0.3 %), ash (7.1 ± 0.5 %), crude proteins (37.7 ± 1.7 %) and crude lipids (0.7 ± 0.1 %). In Song *et al.*(2013)'s paper, Seocheon laver's content of moisture was 7 - 10 %, crude proteins 35 - 38% and crude lipids 1 - 3 %. Wando laver's carbohydrate content was 45.9 ± 1.5%, which was made up of 8.8 ± 0.3 % of moisture, 8.2 ± 0.2 % of ash, 36.2 ± 1.1 % of crude proteins and 0.9 ± 0.4 % of crude lipids. In Hwang (2013)'s paper, Wando laver's content of moisture was 8.6 ± 0.7 %, ash 8.2 ± 0.4 %, crude proteins 36.3 ± 2.0 %

and crude lipids 1.0 ± 0.3 %. Jangheung laver's carbohydrate content was 45.8 ± 3.0 %, which was made up of moisture (8.6 ± 0.7 %), ash (8.2 ± 0.4 %), crude proteins (36.3 ± 2.0 %) and crude lipids (1.0 ± 0.3 %). In Jung et al. (2003)'s paper, Jangheung laver's carbohydrate content was 40.3 %, which was composed of moisture (9.4 %), ash (11.0 %), crude proteins (36.5 %) and crude lipids (0.6 %). The highest value of moisture content, 8.2 ± 0.4 %, was observed in Wando laver, and the least, 8.6 ± 0.7 %, was observed in Jangheung laver. Regarding ash, Jangheung laver showed the highest value of 8.2 ± 0.4 %, and the lowest in Seocheon laver (0.7 ± 0.1 %). For crude proteins, the highest value of 37.7±1.7 % was observed in Seocheon laver, and the lowest value in Wando lever (36.2 ± 1.1 %). Jangheung laver showed the highest value of 1.0 ± 0.3 % for crude laver, and Seocheon laver showed the lowest (0.7 ± 0.1 %). In case of carbohydrate content, Wando laver came in first with 45.9 ± 1.5 %, and Seocheon laver in last with 45.7 ± 2.3 %. As the results of the ANOVA analysis, besides ash, there was no difference between laver from Seocheon and the other two areas regarding the content of moisture, crude proteins, crude lipid and carbohydrates.

2. Mineral Analysis

The result of mineral content analysis is shown in Table 2. There were a total of 12 types of minerals: 26,780.9 ± 144.6 ㎎/㎏ in Seocheon laver; 30,368.2 ± 226.0 ㎎/㎏ in Wando laver; and 27,891.6 ± 139.8 ㎎/㎏ in Jangheung laver, in the order of: Wando>Jangheung>Seocheon. The macro minerals of Seocheon, Wando and Jangheung laver were calcium, potassium, magnesium, sodium and phosphorus, and the average potassium content was 12,597.3 ± 538.6 ㎎/㎏, which made up the largest share. The content of magnesium, sodium and phosphorus was 2,341.7 ± 61.0 ㎎/㎏, 5,244.5 ± 288.9 ㎎/㎏ and 69,801.1 ± 368.0 ㎎/㎏, respectively, where Wando laver showed the highest content. The content of macro minerals contained in laver was in the order of potassium > phosphorus > sodium > magnesium > calcium, but in Hwang (2013)'s paper, the content was in the order of potassium > phosphorus > calcium > magnesium > sodium, which was similar to the result of this paper.

The micro minerals contained in Seocheon and Wando laver were copper, iron, manganese and zinc, and cobalt. Chrome and nickel were not found among them. In case of Jangheung laver, micro minerals, cobalt, chrome, copper, iron, manganese nickel and zinc were found contained within it. Among micro minerals, the content of iron was the highest (125.9 ± 31.0 ㎎/㎏). It was also reported in Seo and Jeong(2007)'s paper that the iron content was the highest among all micro minerals. When micro minerals are compared by production area, the highest copper content was found in Seocheon laver (6.4 ± 0.5 ㎎/㎏), and the highest iron content was found in Wando laver (152.1 ± 13.1 ㎎/㎏). Also, Seocheon laver had the highest manganese (32.9 ± 1.8 ㎎/㎏) and zinc (38.1 ± 2.2 ㎎/㎏) content. According to Hwang (2013)'s paper, the content of micro minerals was in the order of zinc > manganese > copper > nickel > iron = cobalt, which showed a difference with the result of this study: iron >zinc > manganese > copper > nickel > cobalt. Such distinction is thought to be due to the difference in nitric acid response time or sample concentration.

The result of the above experiment showed that Wando and Jangheung laver had higher mineral content than Seocheon laver. This is surmised to be because the coasts of Wando and Jangheung are a half-closed back bay where more inorganic and organic matter flow in than Seocheon,

Table 1. Proximate composition content of *Pyropia dentata*

(%)

	Seocheon	Wando	Jangheung
Moisture	8.8 ± 0.3[1)a2)]	8.8 ± 0.3[a]	8.6 ± 0.7[a]
Ash	7.1 ± 0.5[a]	8.2 ± 0.2[b]	8.2 ± 0.4[b]
Crude proteins	37.7 ± 1.7[a]	36.2 ± 1.1[a]	36.3 ± 2.0[a]
Crude lipid	0.7 ± 0.1[a]	0.9 ± 0.4[a]	1.0 ± 0.3[a]
Carbohydrate	45.7 ± 2.3[a]	45.9 ± 1.5[a]	45.8 ± 3.0[a]

1) Mean±SD(n=5)

2) Values with different superscripts within same row are significantly different at $p<0.001$

Table 2. Mineral content of *Pyropia dentata*

(㎎/㎏)

	Seocheon	Wando	Jangheung
Macro mineral			
Ca	1,544.9 ± 70.0[1)a2)]	2,484.6 ± 124.3[b]	2,541.3 ± 82.8[b]
K	12,046.1 ± 385.2[a]	13,122.3 ± 730.5[b]	12,623.7 ± 446.9[ab]
Mg	2,182.8 ± 85.9[b]	2,341.7 ± 61.0[c]	2,065.8 ± 58.6[a]
Na	4,038.1 ± 169.3[a]	5,244.5 ± 288.9[b]	4,031.6 ± 133.0[a]
P	6,757.9 ± 377.5[ab]	6,980.1 ± 368.0[b]	6,490.1 ± 261.5[a]
Micro mineral			
Co	-	-	0.5 ± 0.1
Cr	-	-	0.6 ± 0.0
Cu	6.4 ± 0.5[c]	4.9 ± 0.3[a]	5.3 ± 0.3[b]
Fe	133.8 ± 13.6[b]	152.1 ± 13.1[c]	91.7 ± 2.1[a]
Mn	32.9 ± 1.9[c]	17.6 ± 1.0[a]	20.2 ± 0.7[b]
Ni	-	-	0.7 ± 0.2
Zn	38.1 ± 2.2[b]	20.5 ± 0.9[a]	20.0 ± 1.0[a]

[1)]Mean±SD(n=10)
[2)]Values with different superscripts within same row are significantly different at $p<0.01$

an open bay. According to MOF (2013)'s research, the aggregate of dissolved inorganic nitrogen (DIN) and dissolved inorganic phosphorus (DIP) was 0.077 ㎎/L on the coast of Boryeong, which is close to Seocheon; 0.095 ㎎/L on the coast of Wando; and 1.00 ㎎/L on the coast of Haenam, which is close to Jangheung.

Table 3. Amino acid content of *Pyropia dentata*

(%)

	Seocheon	Wando	Jangheung
Isoleucine	1.1 ± 0.2[1)NS2)]	1.0 ± 0.0	0.9 ± 0.0
Leucine	2.4 ± 0.4[NS]	2.2 ± 0.0	2.2 ± 0.1
Threonine	0.6 ±0 .1[NS]	0.5 ± 0.0	0.4 ± 0.0
Methionine	0.6 ± 0.1[NS]	0.5 ± 0.0	0.5 ± 0.0
Phenylalanine	1.1 ± 0.2[b3)]	1.0 ± 0.0[ab]	1.0 ± 0.0[a]
Lysine	1.4 ± 0.2[NS]	1.2 ± 0.0	1.3 ± 0.0
Histidine	0.4 ± 0.1[NS]	0.4 ± 0.0	0.4 ± 0.0
Tryptophan	-	-	-
Valine	3.5 ± 0.5[NS]	3.1 ± 0.1	3.1 ± 0.1
Aspartic acid	2.8 ± 0.4[NS]	2.4 ± 0.1	2.5 ± 0.1
Glycine	2.0 ± 0.3[NS]	1.6 ± 0.1	1.6 ± 0.1
Tyrosine	0.6 ± 0.1[NS]	0.5 ± 0.0	0.5 ± 0.0
Serine	1.4 ± 0.2[NS]	1.2 ± 0.0	1.2 ± 0.0
Glutamic acid	3.5 ± 0.5[NS]	3.0 ± 0.1	3.0 ± 0.1
NH3	0.4 ± 0.1[NS]	0.4 ± 0.0	0.4 ± 0.0
Alanine	3.7 ± 0.5[NS]	4.1 ± 0.1	4.2 ± 0.1
Cysteine	-	-	-
Arginine	1.7 ± 0.2[NS]	1.5 ± 0.0	1.5 ± 0.0
Proline	1.0 ± 0.1[NS]	0.9 ± 0.0	0.9 ± 0.0
Total	28.2 ± 0.2	25.5 ± 0.0	25.6 ± 0.0

1) Mean±SD(n=3)
2) Not Significant
3) Values with different superscripts within same row are significantly different at $p<0.05$

3. Amino Acid Analysis

The analysis results of amino acid content are shown in Table 3. A total of 17 types of amino acids were found and the highest content was found in Seocheon (28.2 ± 0.2 %), followed by Wando (25.5 ± 0.0 %) and Jangheung (25.6 ± 0.0 %). The order is in Seocheon>Jangheung> Wando, but there was not so much difference between them. Among the detected amino acids, the content of leucine, valine, aspartic acid, glutamic acid and alanine made up approximately 57.6 % of all content. Also in Lee et al. (2012)'s paper, among amino acids found in Seocheon and Jangheung laver, the content of alanine, glutamic acid, aspartic acid, leucine and valine was high. In particular, unlike other amino acids, the alanine content was higher in Wando and Jangheung laver than in Seocheon laver. According to Shpigel et al. (1999)'s paper, the content of amino acids depends on total nitrogen. In MOF (2013)'s paper, the average total nitrogen in the Wando coastal area was 0.225 ㎎/L; 0.190 ㎎/L in the Goheung coastal area, which is close to Jangheung; and 0.255 ㎎/L in Boryeong, the coastal area which is close to Seocheon. When comparing Shpigel *et al.* (1999)'s and MOF (2013)'s papers, it was identified that the total content of nitrogen was also high in Seocheon, where the highest content of amino acids was found in the laver produced there.

According to the results of this study, it was confirmed that although the experimented laver samples were the same species, their content of proximate composition, minerals and amino acids differed by production site and environment. Also, additional research on the laver's genetics by production area is surmised to be necessary in the future.

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