



Physicochemical characteristics and antioxidant activities of laver cultivars harvested at different times

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Abstract

The aim of this study was to compare the physicochemical characteristics and antioxidant activities of *Pyropia dentata* harvested in November (PD11), *Pyropia seriata* harvested in December (PS12), and *Pyropia yezoensis* harvested in December (PY12), January (PY01), or March (PY03). Total amino acid content (mg/100 g dry wt.) was high: PY01 (41,009.52) > PY12 (40,914.26) > PS12 (35,720.29) > PD11 (33,762.75) > PY03 (32,906.54). The major minerals within the three laver cultivars were Ca, K, Mg, and Na. PD11 exhibited the highest contents of shinorine (2,089.84 mg/100 g dry wt.) and porphyra-334 (3,127.96 mg/100 g dry wt.), which are mycosporine-like amino acids. Of the PY samples harvested at different times, PY03 exhibited the highest contents of shinorine (19.00 mg/100 g dry wt.) and porphyra-334 (438.31 mg/100 g dry wt.). The total phenol content was higher in PD11 (702.99 mg tannic acid equivalent/100 g) than those in the other samples. PD11 had the highest 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid radical-scavenging and ferric reducing antioxidant activities. The results provide basic information regarding the physicochemical characteristics and antioxidant activities of laver cultivars harvested at different times.

Keywords : laver, *Pyropia yezoensis*, *Pyropia seriata*, *Pyropia dentata*, antioxidant activity

Introduction

Laver (*Pyropia* spp.) is the most popular marine red algae that is widely consumed in Korea, along with *Unaria pinnatifida* and *Saccharina japonica*. Laver has been described as “sea clothes” in Gyeongsangdo Jiriji (1424-1425), and cultivated since the late 1400s (Bae et al., 1991). In South Korea, laver is generally cultivated in the Southwestern coastal areas in the winter from November to

March, and approximately 610,000 tons of laver are produced in 2019, which costs about 560 billion KRW. The main varieties of laver produced in Korea are *Pyropia tenera*, *Pyropia yezoensis*, *Pyropia seriata*, and *Pyropia dentata* (Lee et al., 2017). Raw laver is used in soups, salads, and braised dishes or processed as several food products such as dried laver, roasted laver, laver snacks, and seasoned laver to be consumed as side dishes or snacks (Lee et al., 1987).

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Laver is known to be a rich source of fibers, including porphyrin, minerals such as calcium, magnesium, and potassium (Park et al., 2000), and amino acids, including betaine, taurine, aspartic acid, and glutamic acid, but its lipid content is relatively low (<2%) compared to proteins or carbohydrates (Park et al., 2000; Yoshie et al., 1993). Research has shown that it is enriched in lipids especially eicosapentaenoic acid (EPA), which could lower blood cholesterol concentration (Kwon et al., 2018). In addition, mycosporine-like amino acids (MAAs), which are found in marine organisms, are reported to have excellent antioxidant activities and skin protective effects by blocking ultraviolet rays, and porphyra-334 among them has been found in laver (Ying et al., 2019). Many studies have documented the health benefits of laver, including prevention of atherosclerosis (Inoue et al., 2009), cardiovascular disease (Tsuge et al., 2004), and Alzheimer's disease (Liu et al., 2019; Mohibullah et al., 2016), and bone diseases (Ueno et al., 2018), anti-hypertension (Ren et al., 1994; Qu et al., 2010), anti-cancer (He et al., 2019; Kwon et al., 2006; Yu et al., 2015), antioxidant (Zhang et al., 2009; Zhao et al., 2006), anti-inflammatory (Yanagido et al., 2018), and anti-diabetes effects (Chaoet al., 2014; Kitano et al., 2012). With the growing awareness of the health functionality and palatability of laver, demands are steadily rising not only in East Asia but also in Southeast Asia, Europe, and United States. The palatability and health functionality of laver are attributable to the array of constituents of laver. Recently, several studies have investigated the quality properties of laver according to variety, harvest time, and production region (Beak et al., 2019; Kim et al., 2014; Shin et al., 2013), changes in quality during processing and storage (Jo et al., 1995; Son et al., 2014), and the quality characteristics of seasoned laver (Jeon et al., 2008; Seo et al., 2007). Moreover, the nutritional composition and bioactive compounds content of the laver vary greatly according to the variety, cultivation, harvest time, and oceanic conditions. Thus, more systematic studies are needed to examine the quality characteristics of laver.

In recent, three laver varieties are widely cultivated in Jeonnam, the primary region of laver production in Korea. *P. dentata* is produced from November to December, *P. yezoensis* in December, and *P. seriata* from December to March. Thus, this study aims to present basic data for laver

processing by comparing the physicochemical characteristics and antioxidant activities of the specimens of five varieties harvested at different times, including *P. dentata*, *P. seriata*, and *P. yezoensis*.

Materials and method

Experimental materials and reagents

The laver used in this experiment was provided by Jeollanam-do Institute of Ocean & Fisheries Science, Haenam office. *P. dentata* was harvested in November 2019 in Imhado, Haenam, and *P. seriata* was harvested in December 2019 in Imhado, Haenam. *P. yezoensis* was harvested in December 2019 and January and March 2020 in Eoran, Haenam. The samples were freeze-dried (PVTFD50R, Ilsin, Dongducheon, Korea) and stored at -40°C before analysis. The Folin-Ciocalteu's phenol reagent, sodium carbonate (Na_2CO_3), tannic acid, 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABST^+), potassium persulfate, 2,4,6-tris(2-pyridyl)-*S*-triazine (TPTZ), iron (II) sulfate (FeSO_4), and ferric chloride hexahydrate were purchased from Sigma-Aldrich Chemical Co. (St. Louis, MO, USA). Mycosporine-like amino acids (shinorine and porphyra-334) used in this study were isolated from laver at our laboratory. Other organic solvents and reagents were high performance liquid chromatography (HPLC) grade or guaranteed reagent.

Total amino acid content

Total amino acid content was analyzed using the protocol used by Seo et al. (2007). 10 mL of 6 N hydrogen chloride (HCl) was added to 50 mg of freeze-dried laver, flushed with nitrogen gas, and heated at 105°C for 24 h using a heating block. The mixture was then filtered using a 3G-4 glass filter, and the filtrate was concentrated under reduced pressure using a rotary vacuum evaporator (N-1200B, Eyela, Tokyo, Japan). The concentrate was dissolved with sodium 10 mL of citrate buffer solution (pH 2.2), filtered through a 0.2 μm membrane filter (Satorius Stedim Biotech, Goettingen, Germany), and analyzed using an automated amino acid analyzer (S433-H SYKAM, Eresing, Germany) equipped with a cation separation column (LCA K06/Na, 4.6 \times 150 mm, 7 μm , Sykam, Eresing, Germany). The flow rate of the buffer solution (pH 3.45-10.85) was set at 0.45

mL/min, and that of reagent was set at 0.25 mL/min. A fluorescence detector ($E_x=440$ nm, $E_m=570$ nm) was used for amino acid detection.

Mineral content

Mineral content was analyzed in accordance with the Food Codex (Ministry of Food and Drug Safety, 2020). Ten milliliter of nitric acid was added to 0.3 g of freeze-dried laver sample in a Teflon-coated vessel, and the mixture was dissolved in microwave (MARS, CEM, Matthews, NC, USA). The dissolved solution was diluted to 50 mL using distilled water and analyzed with an inductively coupled plasma atomic emission spectrometer (Optima 8300, PerkinElmer, Waltham, MA, USA).

Shinorine and porphyrin-334 content

Extraction was performed for 24 h by adding 200 mL of 50% methanol (MeOH) to 3 g of freeze-dried laver at room temperature. The extract was filtered through a No. 2 filter paper (Whatman, Maidstone, England) to obtain the filtrate. Two hundred milliliter of 50% MeOH was added to the residual sample and a second filtrate was obtained via the same protocol. The two filtrates were mixed and concentrated under a vacuum to obtain 50% MeOH extract. This extract was dissolved by adding 20 mL of MeOH and filtered through a 0.45 μ m membrane filter (Advantec, Tokyo, Japan). Shinorine and porphyrin-334 were analyzed using a HPLC (SPD-M20D, Shimadzu) equipped with a Capcell pak C₁₈ AQ column (4.6 \times 250 mm, 5 μ m, Shiseido, Tokyo, Japan) and a photodiode array (PDA, 190-800 nm) detector. The mobile phase consisted of water containing 0.1% acetic acid (A) and acetonitrile (B) with using a gradient elution (0-5 min 0% B; 5-25 min 0-20% B; 25-30 min 20% B). The compounds were monitored at 330 nm and the flow rate of the mobile phase was set at 1 mL/min. Shinorine and porphyrin-334 purified from laver were used as the external standards.

Total phenol content

In accordance with Son's (2014) method, extraction was performed for 24 h by adding 50 mL of distilled water to 2 g of freeze-dried laver and filtered through a suction filter (No. 2, Whatman, Maidstone, England) to obtain the filtrate. The filtrate was concentrated under reduced pressure and

prepared to a concentration of 200 mg/mL by adding distilled water. Total phenolic content was measured using the Folin-Denis method (Folin and Denis, 1912), which utilizes the principle in which the reduction of the Folin-Ciocalteu's phenol reagent by phenolic compounds generates the molybdenum blue. In other words, 20 μ L of Folin-Ciocalteu's reagent was added to 20 μ L of the sample and incubated in a dark room for 3 min. After adding 200 μ L of 10% Na₂CO₃ to this solution and incubating in a dark room for 15 min, absorbance was measured at 765 nm using a microplate reader (Infinite M200 PRO, Tecan, Salzburg, Austria). The total phenol content of the sample was quantified from the absorbance value using a standard curve obtained using tannic acid and was expressed as mg tannic acid equivalent per gram of laver sample (mg TAE/g dry wt.).

ABTS⁺ radical-scavenging activity

The ABTS⁺ radical-scavenging activity of the aqueous laver extract prepared above was measured using the method delineated by Pellegrin et al. (1999). In other words, 5 mL of 7 mM ABTS⁺ solution was mixed with 88 μ L of 140 mM potassium persulfate solution and incubated in dark for 14-16 h. Then, absolute ethanol was added to prepare an ABTS⁺ radical solution with an absorbance of 0.70 \pm 0.02 at 734 nm. 20 μ L of the sample solution was mixed with 180 μ L of ABTS solution and incubated in dark for 3 min, and absorbance was read at 734 nm using a microplate reader (Infinite M200 PRO, Tecan). The ABTS⁺ radical-scavenging activity was measured using the equation below with the absorbance values of the non-treated and treated samples. Further, the concentration at 50% ABTS⁺ radical-scavenging activity (SC₅₀, mg/mL) was calculated using the free radical-scavenging activity curve by concentration for each sample.

$$\begin{aligned} & \text{ABTS}^+ \text{ radical-scavenging activity (\%)} \\ & = (1 - \text{Sample absorbance} / \text{Control absorbance}) \times 100 \end{aligned}$$

Ferric ion reducing antioxidant power (FRAP)

FRAP activity was measured using the method proposed by Benzie and Strain (1996). The FRAP reagent was prepared by heating 40 mL acetate buffer (300 mM, pH 3.6) at 37°C and mixing with 4 mL of 10 mM TPTZ dissolved in 40 mM HCl and 4 mL of 20 mM ferric chloride hexahydrate (FeCl₃ · 6H₂O). 20 μ L of the aqueous laver

extract was added to 180 μ L of the prepared FRAP reagent and incubated for 10 min at 37°C. Absorbance was measured at 593 nm using a microplate reader (Infinite M200 PRO, Tecan). The reducing power was computed from these absorbance values using a standard curve for iron (II) sulfate at concentrations of 0.156, 0.313, 0.625, 1.25, and 2.5 mM.

Statistical analysis

All experiments were conducted three times, and the data were statistically analyzed using IBM SPSS statistics (24, IBM Corp., Armonk, NY, USA). The results were presented as mean and standard deviation. One-way ANOVA and post-hoc test (Scheffe test) were performed to analyze the

differences among the samples, and significance was set at $p < 0.05$.

Results and discussion

Total amino acid content

Table 1 shows the total amino acid content of laver samples with varying laver varieties and harvest periods. Total amino acid content was the highest in *P. yezoensis* (PY) harvested in December (PY12, 40,914.26 mg/100 g dry wt.) and January (PY01, 41,009.52 mg/100 g dry wt.), respectively and the lowest in *P. dentata* (PD11, 33,762.75 mg/100 g dry wt.). Total amino acid content of laver varied

Table 1. Total amino acid content in three laver cultivars harvested at different times

Compounds	Contents (mg/100 g dry wt.)				
	PD11 ¹⁾	PS12	PY12	PY01	PY03
Isoleucine	1,270.83±182.04 ^b	1,469.50±119.69 ^b	1,821.87±52.66 ^a	1,862.45±191.40 ^a	1,404.46±50.35 ^b
Leucine	2,662.35±340.85 ^b	3,039.99±189.57 ^{ab}	3,428.10±59.36 ^a	3,478.73±377.88 ^a	2,751.09±48.24 ^b
Lysine	1,863.59±256.37 ^c	2,056.51±160.52 ^{abc}	2,289.17±148.26 ^{ab}	2,324.78±337.43 ^a	1,902.28±19.22 ^{bc}
Methionine	693.28±78.15 ^b	572.06±53.59 ^b	1,072.20±105.34 ^a	968.35±24.93 ^a	708.94±151.11 ^b
Phenylalanine	1,398.19±175.00 ^c	1,619.99±114.96 ^{bc}	1,938.14±48.53 ^a	1,818.41±183.78 ^{ab}	1,473.65±10.59 ^c
Threonine	1,909.88±311.16 ^(a4)5)	2,141.56±59.78 ^{ab}	2,351.08±40.42 ^a	2,332.09±357.09 ^{ab}	1,919.80±90.20 ^b
Valine	2,235.56±276.18 ^a	2,507.00±168.97 ^{ab}	2,896.39±88.33 ^a	2,862.54±338.62 ^a	2,239.84±53.92 ^b
Total EAA ²⁾	12,033.67±1,540.51 ^b	13,406.62±817.57 ^b	15,796.93±215.15 ^a	15,647.36±1,777.20 ^a	12,400.05±259.81 ^b
Alanine	4,338.85±720.15 ^a	3,377.89±247.50 ^b	3,679.59±175.36 ^{ab}	3,737.37±399.96 ^{ab}	3,302.79±50.40 ^b
Arginine	2,728.01±579.28 ^a	2,966.93±184.65 ^a	3,255.8±290.84 ^a	3,280.88±651.37 ^a	2,579.90±204.81 ^a
Aspartic acid	3,388.30±571.55 ^a	3,823.29±151.65 ^a	4,245.11±163.42 ^a	4,346.54±868.94 ^a	3,490.59±278.55 ^a
Cystine	35.17±60.91 ^b	11.55±20.00 ^b	244.6±63.31 ^a	190.54±69.02 ^a	63.10±63.84 ^b
Glutamic acid	3,738.66±754.73 ^a	3,797.10±103.29 ^a	4,177.62±119.71 ^a	4,377.63±750.33 ^a	3,643.63±159.75 ^a
Glycine	2,098.91±374.58 ^b	2,160.16±100.60 ^{ab}	2,630.82±50.61 ^a	2,570.99±396.24 ^{ab}	2,095.64±79.39 ^b
Histidine	529.17±30.71 ^a	634.78±167.63 ^a	660.65±108.85 ^a	552.68±68.07 ^a	480.33±75.37 ^a
Proline	1,731.41±390.01 ^a	2,062.57±123.95 ^a	2,251.63±339.18 ^a	2,306.52±688.38 ^a	1,783.59±336.24 ^a
Serine	1,944.08±345.15 ^a	2,086.53±55.21 ^a	2,305.40±43.72 ^a	2,355.39±411.74 ^a	1,881.46±83.09 ^a
Tyrosine	1,196.53±173.06 ^b	1,392.86±124.98 ^b	1,666.11±67.02 ^a	1,643.62±163.71 ^a	1,185.44±49.38 ^b
Total AA ³⁾	33,762.75±5,374.50 ^b	35,720.29±1,495.48 ^{ab}	40,914.26±405.28 ^a	41,009.52±6,071.13 ^a	32,906.54±859.96 ^b

¹⁾PD11, *P. dentata* harvested in November; PS12, *P. seriata* harvested in December; PY12, *P. yezoensis* harvested in December; PY01, *P. yezoensis* harvested in January; PY03, *P. yezoensis* harvested in March.

²⁾Total essential amino acid.

³⁾Total amino acid.

⁴⁾All values are mean±SD in triplicates.

⁵⁾Different letters (^{a-d}) in the same column mean significantly different ($p < 0.05$).

greatly not only according to the variety but also according to the harvest times. In our analysis of the total amino acid contents of PY harvested at different times, the sample collected in March (PY03, 32,906.54 mg/100 g dry wt.) had a lower total amino acid content than the samples collected in December (PY12, 40,914.26 mg/100g dry wt.) and January (PY01, 41,009.52 mg/100 g dry wt.). This is consistent with previous results that the total amino acid contents of laver varies widely according to the growing environment, such as season, habitat, and oceanic conditions (Noda, 1993). In particular, PY had a higher total amino acid contents when harvested in December or January compared to March, suggesting that the abundance of amino acids in the growing environments of December and January is higher than that in March.

The most abundant amino acids in all laver varieties were aspartic acid, glutamic acid, leucine, arginine, and alanine, and these five amino acids accounted for 46.24-50.78% of the total amino acid content. This is similar to the major amino acid composition of laver reported in the study on dried laver produced in Korea by Lee et al. (1987) and that of the study on the nutritional composition of commercial laver products by Kim et al. (2014). Essential amino acids, such as threonine, valine, methionine, isoleucine, leucine, phenylalanine, and lysine, were some of the major ingredients of laver. The percentage of essential amino acids from the total amino acid content was 37.68-38.61% in PY,

37.53% in PS12, and 35.64% in PD11, with no marked differences across varieties. Leucine, which is a branched chain amino acid (BCAA), was the most abundant in all laver varieties, and laver contained a rich amount of essential BCAAs other than leucine, such as valine and isoleucine. In other words, BCAA content was 6,395.39-8,203.72 mg/100 g dry wt. in PY, 7,016.49 mg/100 g dry wt. in PS12, and 6,168.74 mg/100 g dry wt. in PD11, accounting for 17.65-21.05% of total amino acid contents. BCAAs is not only an important regulator of insulin signaling and lipid metabolism but also is the largest storage of muscle proteins that plays a crucial role in protein metabolism (Yao et al., 2016). Particularly, leucine has been reported to enhance protein synthesis and inhibit protein degradation (Kimball and Jefferson, 2001). Laver is an important source of amino acids, and with its rich content of BCAAs, including leucine, valine, and isoleucine, it has benefits in terms of insulin signaling, lipid metabolism, enhanced protein synthesis, and suppressed protein degradation.

Mineral contents

Table 2 shows the mineral contents of laver samples with varying laver varieties and harvest periods. The major minerals found in laver were calcium (Ca), potassium (K), magnesium (Mg), and sodium (Na), all of which accounted for 96.0-99.2% of total mineral content. This is consistent

Table 2. Mineral contents in three laver cultivars harvested at different times

Samples	Mineral contents (mg/100 g dry wt.)				
	PD11 ¹⁾	PS12	PY12	PY01	PY03
K	887.31±28.89 ^a	121.03±3.37 ^d	345.83±14.94 ^c	372.47±12.68 ^{bc}	463.55±12.96 ^b
Ca	493.91±3.69 ^{2)a3)}	485.57±4.14 ^a	363.44±6.25 ^b	238.87±15.28 ^c	235.32±7.25 ^c
Mg	467.50±10.07 ^a	492.71±9.97 ^a	383.59±14.61 ^b	357.11±9.02 ^b	374.68±2.52 ^b
Na	351.96±10.90 ^b	279.21±7.03 ^c	387.96±13.28 ^a	407.83±11.96 ^a	272.29±5.94 ^c
Fe	7.50±0.45 ^b	24.49±3.47 ^a	17.51±2.35 ^a	20.20±5.36 ^a	17.97±1.82 ^a
Zn	4.69±0.20 ^c	10.55±0.36 ^a	5.59±0.13 ^b	4.91±0.37 ^{bc}	4.58±0.08 ^c
Mn	2.47±0.06 ^a	1.28±0.09 ^d	1.70±0.07 ^c	1.91±0.09 ^{bc}	2.06±0.03 ^b
Cu	0.38±0.02 ^d	1.56±0.06 ^a	1.31±0.06 ^b	1.07±0.05 ^c	0.94±0.03 ^c

¹⁾PD11, *P. dentata* harvested in November; PS12, *P. seriata* harvested in December; PY12, *P. yezoensis* harvested in December; PY01, *P. yezoensis* harvested in January; PY03, *P. yezoensis* harvested in March.

²⁾All values are mean±SD in triplicates.

³⁾Different letters (^{a-d}) in the same column mean significantly different (p<0.05).

with previous findings that Ca, K, Mg, and Na were the primary minerals found in commercial laver products and PY in different regions (Kim et al., 2014; Mok et al., 2011).

The results of this study showed that the types and contents of minerals varied widely according to variety and harvest time. Although the mineral content could not be compared among laver varieties due to the varying harvest periods, PD11 had the richest K content, while PS12 had relatively higher amounts of Ca and Mg. PY had high contents of Ca, K, Mg, and Na at all harvest periods, although the specific contents of minerals differed slightly depending on the harvest period.

Regarding trace minerals in laver, iron (Fe) was found in the highest concentration at 7.50-24.49 mg/100 g dry wt. In particular, Fe content was twofold higher in PS12 (24.49 mg/100 g dry wt.) and PY (17.51-20.20 mg/100 g dry wt.) than in PD11 (7.50 mg/100 g dry wt.), with no statistically significant difference in Fe content between PS12 and PY ($p>0.05$). Fe content also did not statistically significantly differ in PY samples according to the harvest period. Interestingly, zinc (Zn) content varied more greatly according to laver variety and harvest period. Zn content was more than 1.8 times higher in PS12 (10.55 mg/100 g dry wt.) than in PD11 (4.69 mg/100 g dry wt.) and PY (4.58-5.59 mg/100 g dry wt.). Zn content in PY was similar to that in PD, and although there were significant differences in Zn content according to harvest period, the content was very low compared to that in PS12 and thus was insignificant. Manganese (Mn) content was the highest in PD11 (2.47 mg/100 g dry wt.), while copper (Cu) content was the highest in PS12 (1.56 mg/100 g dry wt.).

Kim et al. (2014) and Jung et al. (2016) reported that mineral contents differ even in the same variety depending on the environment of the farming region. Although the exact order of mineral content differed, they found that the major minerals were Ca, K, Mg, and Na, with Fe being the most abundant trace mineral. Baek et al. (2019) reported that PY and PD differ in their mineral contents, and similar to the results of this study, the major minerals were Ca, K, Mg, and Na, with Fe being the most abundant trace mineral. In contrast with our findings, Fe content was 1.7 times higher in PD. This may be attributable to the differences in the farming environments.

Shinorine and porphyra-334 content

MAAs, mycosporine-derived compounds found in various marine organisms, including red algae, consist of a cyclohexenimine or cyclohexenone ring with various substituents such as amino acids and amino alcohol (Carreto et al., 2011). MAAs have been reported to have antioxidant effects and protective effects against ultraviolet rays and photooxidative stress (Shick et al., 2002; Venkatraman et al., 2019; Ying et al., 2019). Particularly, shinorine and porphyra-334 isolated from microalgae not only protects against cytotoxicity caused by UV irradiation but also suppresses the expression of COX-2, an inflammation-related gene (Cho et al., 2014; Jung et al., 2014). A recent study reported that laver contains MAAs, including shinorine and porphyra-334 (Ying et al., 2019), and these compounds have surfaced as some of the beneficial components of laver. Thus, the shinorine and porphyra-334 contents of three laver varieties were analyzed via ODS-HPLC. As shown in Table 3, the shinorine content was markedly higher in PD11 (2,090 mg/100 g dry wt.) harvested in November compared to that of PY (1.56-19.00 mg/100 g dry wt.) and PS12 (1.62 mg/100 g dry wt.). Further, the porphyra-334 content was also markedly higher in PD11 (3,128 mg/100 g dry wt.) compared to that of PY (3.02-438.31 mg/100 g dry wt.) and PS12 (2.17 mg/100 g dry wt.). Among PY samples with varying harvest periods, the sample collected in March (PY03) had markedly higher shinorine (19.00 mg/100 g dry wt.) and porphyra-334 content

Table 3. Shinorine and porphyra-334 contents in three laver cultivars harvested at different times

Samples	Content (mg/100 g dry wt.)	
	Shinorine	Porphyra-334
PD11 ¹⁾	2,090±107 ^{2)a3)}	3,128±154 ^a
PS12	1.62±0.22 ^c	2.17±0.07 ^d
PY12	1.56±0.01 ^c	3.02±0.11 ^d
PY01	1.56±0.01 ^c	17.02±0.07 ^c
PY03	19.00±0.43 ^b	438.31±4.83 ^b

¹⁾PD11, *P. dentata* harvested in November; PS12, *P. seriata* harvested in December; PY12, *P. yezoensis* harvested in December; PY01, *P. yezoensis* harvested in January; PY03, *P. yezoensis* harvested in March.
²⁾All values are mean±SD in triplicates.

³⁾Different letters (^{a-d}) in the same column mean significantly different ($p<0.05$).

(438.31 mg/100 g dry wt.) compared to samples collected in December (PY12) and January (PY01). In essence, shinorine and porphyra-334 have various biological activities, including skin protection, anti-inflammatory action, and antioxidant activities, as previously mentioned, and PD that contains large amounts of these compounds is promising as an ingredient for functional food compared to other laver varieties. Furthermore, PY03 had a higher shinorine and porphyra-334 content than PY12 and PY01, so it would be a more useful ingredient in various aspects.

Total phenol content

Phenolic compounds, such as flavonoids, anthocyanins, nignans, and stilbenes, are secondary metabolites widely found in plants (Lin et al., 2016). Phenolic compounds exhibit an array of biological activities, including antioxidant activity, dental caries prevention, hypertension prevention, and anti-cancer effects (Yu et al., 2006). While laver was not a rich source of various phenolic compounds, it has been reported to contain catechol, rutin, catechin, and quercetin (Kazłowska et al., 2010). In addition, it was reported to have increased the total phenol content of steamed fish cake and crispy rice snacks when adding laver powder (Kim et al., 2020a; Kim et al., 2020b). Phenolic compounds are expected to contribute to the health functionalities of laver. Therefore, this study investigated the total phenol content of laver samples from different varieties and harvested at varying periods, and the results are shown in Table 4. By variety, PD11 had the highest total phenolic content (702.99

mg TAE/100 g dry wt.), followed by PY (136.82-184.14 mg TAE/100 g dry wt.), and PS12 had the lowest phenol content (50.12 mg TAE/100 g dry wt.). In terms of harvest periods, PY12 had significantly higher phenolic content (184.14 mg TAE/100 g dry wt.) compared to PY01 (136.82 mg TAE/100 g dry wt.) and PY03 (144.32 mg TAE/100 g dry wt.). These results supported that total phenol content of laver harvested in December had a higher total phenol content than laver harvested in January to March (Kang et al., 2018). These results show that the total phenol content of laver varies according to the variety and harvest period. Particularly, PD can be utilized to develop food products due to a higher total phenol content than PS and PY, which enhanced functional ingredients, such as phenolic compounds. However, the structures of these phenolic compounds need to be confirmed even though PD is suggested to contain diverse phenolic compounds, including catechol, rutin, and hesperidin found in PY.

ABTS⁺ radical-scavenging activity and FRAP

ABTS⁺ radical-scavenging activities of laver extracts were examined by concentration, and the concentrations at 50% radical-scavenging activity (SC₅₀) were computed (Table 5). PD11 had the lowest SC₅₀ (55.40 mg/mL), followed by PY (68.58-144.51 mg/mL) and PS12 (192.76 mg/mL), which shows that PD has the highest radical-scavenging potential. In addition, PY samples showed varying levels of ABTS⁺ radical-scavenging depending on the harvest period. This means that PY12 had the highest radical-scavenging activity

Table 4. Total phenolic contents in three laver cultivars harvested at different times

Samples	Total phenolic contents (mg TAE/100 g)
PD11 ²⁾	702.99±4.00 ^{2)a3)}
PS12	50.12±2.88 ^d
PY12	184.14±7.51 ^b
PY01	136.82±4.01 ^c
PY03	144.32±1.75 ^c

¹⁾PD11, *P. dentata* harvested in November; PS12, *P. seriata* harvested in December; PY12, *P. yezoensis* harvested in December; PY01, *P. yezoensis* harvested in January; PY03, *P. yezoensis* harvested in March.

²⁾All values are mean±SD in triplicates.

³⁾Different letters (^{a-d}) in the same column mean significantly different (p<0.05).

Table 5. ABTS⁺ radical-scavenging activity of three laver cultivars harvested at different times

Samples	ABTS ⁺ radical-scavenging activity (SC ₅₀ , mg/mL)
PD11 ¹⁾	55.40±2.97 ^c
PS12	192.76±6.81 ^{2)a3)}
PY12	68.58±1.70 ^d
PY01	119.91±4.43 ^c
PY03	144.51±2.11 ^b

¹⁾PD11, *P. dentata* harvested in November; PS12, *P. seriata* harvested in December; PY12, *P. yezoensis* harvested in December; PY01, *P. yezoensis* harvested in January; PY03, *P. yezoensis* harvested in March.

²⁾All values are mean±SD in triplicates.

³⁾Different letters (^{a-d}) in the same column mean significantly different (p<0.05).

at $SC_{50}=68.58$ mg/mL, while PY3 had the lowest radical-scavenging activity at $SC_{50}=144.51$ mg/mL. Kim et al. (2015) demonstrated that marine algae with a high total phenolic content also had superior ABTS radical-scavenging activity, which was similar to this study.

FRAP was measured for laver extracts with varying varieties and harvest periods, and the results are shown in Table 6. The FRAP results were similar to the ABTS⁺ radical-scavenging activities. In other words, PD11 showed the highest ABTS⁺ radical-scavenging activity and also had the highest FRAP (1.47 mM), while PS12 had the lowest FRAP (0.29 mM). Further, the FRAP of PY varied according to the harvest period, similar to the results of ABTS⁺ radical-scavenging activity. PY12 had the highest FRAP (1.19 mM), while there were no significant differences in FRAP between PY01 (0.75 mM) and PY03 (0.70 mM).

Antioxidant in laver include pigments such as chlorophyll and carotenoids as well as polyphenols and porphyrin, and the level of antioxidant activity differs according to the production region and antioxidant compounds (Kang et al., 2018; Oh et al., 2013). PD has superior antioxidant activities to PS and PY and thus is promising as an ingredient for natural antioxidants and health functional products.

In conclusion, we examined the physicochemical properties and antioxidant activities of *Pyropia yezoensis* (PY), *Pyropia seriata* (PS), and *Pyropia dentata* (PD), primarily produced in Jeonnam. PY produced in December (PY12) and January (PY01) had higher total amino acid content (40,914.26 mg/100 g dry wt.) than PS12 (35,720.29 mg/100 g dry wt.) and PD 11 (33,762.75 mg/100 g dry wt.).

According to harvest time, PY01 had the highest total amino acid content (41,009.52 mg/100 g dry wt.), while PY03 had the lowest total amino acid content (32,906.54 mg/100 g dry wt.). This is consistent with previous reports that the total amino acid content of laver varies greatly according to the growing environment, such as season, habitat, and oceanic conditions. The major minerals found in laver were Ca, K, Mg, and Na, and Fe was the most abundant trace mineral (7.50-24.49 mg/100 g dry wt.). Mineral contents also seem to differ according to the variety and harvest period which depend on the environments of the cultivation regions. Shinorine and porphyrin-334 contents were markedly higher in PD11 (2,089.84 mg/100 g dry wt. and 3,127.96 mg/100 g dry wt., respectively) compared to the other two varieties. As shinorine and porphyrin-334 have skin-protective, anti-inflammatory, and antioxidant effects, PD11 is considered a promising ingredient for functional foods. By the harvest time, the shinorine and porphyrin-334 contents were markedly higher in PY03 (19.00 mg/100 g dry wt. and 438.31 mg/100 g dry wt., respectively) than PY12 or PY01, suggesting that PY harvested in March should be utilized if PY is used as a functional food ingredient. PD11 showed the highest total phenol content (702.99 TAE mg/100 g dry wt.), followed by PY (136.82-184.14 TAE mg/100 g dry wt.), and PS12 (50.12 TAE mg/100 g dry wt.). PD11 also exhibited higher ABTS radical-scavenging activity and FRAP than PS12 and PY, suggesting that it has superior antioxidant activities to the other two varieties.

요 약

본 연구에서는 전남지역에서 주로 생산되고 있는 잇바디돌김, 모무늬돌김, 그리고 방사무늬김을 대상으로 이화학적 특성 및 항산화 활성을 조사하였다. 김의 구성아미노산 함량은 12월과 1월에 채취한 방사무늬김이 40,914.26 mg/100 g dry wt.의 이상으로 모무늬돌김(PS12, 35,720.29 mg/100 g dry wt.)과 잇바디돌김(PD11, 33,762.75 mg/100 g dry wt.)에 비해 상대적으로 높은 함량이었다. 채취시기가 다른 방사무늬김의 총 구성아미노산 함량을 비교한 결과, 1월에 채취한 시료(PY01, 41,009.52 mg/100 g dry wt.)에서 가장 높았던 반면, 3월에 채취한 시료(PY03, 32,906.54 mg/100 g dry wt.)로 가장 낮았다. 이는 김의 총아미노산 함량이 계절, 생육장소, 해황 등 생육환경에 따라 그 변동이 심한 것으로 보고된 결과와 일치하였다. 김에 함유된 주요 무기질은 Ca, K, Mg,

Table 6. Ferric reducing antioxidant power (FRAP) of three laver cultivars harvested at different times

Samples	FRAP (FeSO ₄ , mM)
PD11 ¹⁾	1.47±0.05 ^{2)a3)}
PS12	0.29±0.01 ^d
PY12	1.19±0.93 ^b
PY01	0.75±0.04 ^c
PY03	0.70±0.04 ^c

¹⁾PD11, *P. dentata* harvested in November; PS12, *P. seriata* harvested in December; PY12, *P. yezoensis* harvested in December; PY01, *P. yezoensis* harvested in January; PY03, *P. yezoensis* harvested in March.

²⁾All values are mean±SD in triplicates.

³⁾Different letters (^{a-d}) within a column are significantly different ($p<0.05$).

Na였으며, 미량 무기질은 Fe가 7.50-24.49 mg/100 g dry wt 로 가장 높은 함량이었다. 이는 양식지역의 환경에 따라 품종 및 채취시기별로 차이를 보이는 것으로 사료된다. Shinorine 과 porphyra-334는 잇바디돌김(PD11)이 2,090 mg/100 g dry wt., 3,128 mg/100 g dry wt.으로 두 종에 비해 월등히 높은 함량을 보였다. 잇바디돌김은 다른 두 품종에 비해 피부보호, 항염증, 항산화 활성 등의 생리활성을 나타내는 shinorine과 porphyra-334를 다량 함유하고 있어 기능성 식품 소재로의 활용이 기대된다. 채취시기별 방사무늬김의 shinorine과 porphyra-334 함량은 3월에 채취된 방사무늬김(PY03)이 각각 19.00 mg/100 g dry wt., 438.31 mg/100 g dry wt.로 12월, 1월에 채취된 방사무늬김보다 월등히 높아 방사무늬김을 기능성 식품소재로 사용한다면 이를 이용하는 것이 용이할 것으로 판단된다. 총폴리페놀 함량은 잇바디돌김(PD11, 702.99 TAE mg/100 g dry wt.) > 방사무늬김(PY, 136.82-184.14 TAE mg/100 g dry wt.) > 모무늬돌김(PS12, 50.12 TAE mg/100 g dry wt.) 순서였다. ABTS radical 소거활성과 철환원력(FRAP) 측정 결과, 잇바디돌김(PD11)은 모무늬돌김(PS12)와 방사무늬김(PY)에 비해 높은 항산화 활성을 나타냈다.

Conflict of interests

The authors declare no potential conflict of interest.

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