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# Effects of Extraction and Processing Methods on Antioxidant Compound Contents and Radical Scavenging Activities of Laver (*Porphyra tenera*)

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**ABSTRACT:** Laver is one of the most consumed edible red algae seaweeds in the genus *Porphyra*. Laver is primarily prepared in the form of dried, roasted, and seasoned products. We investigated the total polyphenol and flavonoid contents of laver products, and evaluated the *in vitro* antioxidant properties of solvent extracts from commercially processed laver products. Significant differences in the concentration of phenolic compounds were found among differently processed laver. The total phenolic content for laver extracts ranged from 10.81 mg gallic acid equivalent (GAE)/g extract to 32.14 mg GAE/g extract, depending on extraction solvent and temperature. Laver extracts contained very few flavonoids (0.55 mg catechin equivalent/g extracts to 1.75 mg catechin equivalent/g extracts). 2,2-Diphenyl-1-picrylhydrazyl (DPPH), 2,2'-azino-bis-3-ethylbenzothiazoline-6-sulphonic acid (ABTS), hydroxyl radical, and superoxide anion scavenging assays were used to determine the radical scavenging capacities of laver extracts. These assays revealed that the processing method and extraction condition affected the antioxidant potentials of laver. Antioxidant activity of dried laver, roasted laver, and seasoned laver increased in a concentration-dependent manner (100~1,000 μg/mL). The radical scavenging activities of 37°C and 100°C water extracts were lower than that of a 37°C 70% ethanol extract. The highest radical scavenging capacity was observed in the 37°C 70% ethanol extracts of dried laver, roasted laver, and seasoned laver. Overall, these results support that notion that laver contains bioactive compounds, such as polyphenols and flavonoids, which may have a positive effect on health.

**Keywords:** laver, dried, roasted, seasoned, antioxidant

## INTRODUCTION

Laver (*Porphyra tenera*) is traditionally consumed in Asia, particularly in Korea, Japan, and China, but is only occasionally consumed in other parts of the world (1). However, the increasing popularity of oriental cuisine in Western countries in recent years has increased the demand for this marine vegetable. Laver is characterized by high concentrations of fiber and minerals, a low fat content, and, in some cases, relatively high protein levels (2-4). Laver contains various kinds of inorganic and organic substances, such as polyphenols, carotenoids, and tocopherols that benefit human health (5). Consumption of seaweeds, including laver, increases the intake of dietary fiber and lowers the occurrence of some chronic diseases such as diabetes, obesity, heart disease, and cancer (6). Recent studies have reported that seaweed extracts have strong antioxidant properties (7,8).

Red seaweed, including laver, is considered a rich source of antioxidants, such as polyphenols, phlorotannins, and fucoxanthin (9,10). One study reported that extracts isolated from various red seaweeds have antioxidant activities and contain phenolic compounds (11). Lim et al. (12) reported that total phenolic content, flavonoids, chlorophyll, and carotenoids found in seaweed may contribute to its antioxidant activity.

Laver products are popular side dishes in Asian countries where rice is the staple food. Korea, Japan, and China are by far the largest consumers of laver (13). Each year, six million tons of fresh algae are cultivated worldwide, comprising about 90% of the commercial demand (13). Laver is mostly typically prepared as a dried, roasted, or seasoned product. Dried laver is prepared from raw laver through various processes. Roasted laver and seasoned laver products are manufactured by roasting or seasoning (usually with sesame oil and salt) dried

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laver, respectively. Dried laver is considered a semi-processed product of roasted or seasoned laver, as well as a processed product for direct consumption (14). Dried or roasted laver is consumed as a main ingredient in gimbap, sushi, or steamed rice rolled with various ingredients.

Despite the fact that most laver is manufactured and consumed in a processed form, few studies have investigated the antioxidant compounds contained in processed laver. In addition, little information is available regarding the relationships between the active compounds and antioxidant activities of differently processed laver. Thus, we evaluated the *in vitro* antioxidant properties of solvent extracts from commercially processed laver products and correlated their antioxidant activities with 2,2-diphenyl-1-picrylhydrazyl (DPPH), 2,2'-azino-bis-3-ethylbenzothiazoline-6-sulphonic acid (ABTS), hydroxyl, and superoxide radical scavenging activities. In addition, we determined the total polyphenol and flavonoid contents from the differently processed laver extracts.

## MATERIALS AND METHODS

### Chemicals

Folin-Ciocalteu's phenol reagent, DPPH, ABTS, gallic acid, catechin, ascorbic acid, nitro blue tetrazolium chloride (NBT), nicotinamide adenine dinucleotide (NADH), Tris-HCl, potassium hexacyanoferrate, trichloroacetic acid, ferric chloride, and para-methyl styrene (PMS) were purchased from Sigma-Aldrich (St. Louis, MO, USA). Organic solvents were purchased from Honeywell Burdick & Jackson (Muskegon, MI, USA). All reagents and chemicals used were of analytical grade.

### Laver preparation

Laver that had been collected from Wando (Jeonnam, Korea) and dried in December of 2012 was purchased from a local market. To prepare differently processed laver, we followed the usual laver cooking methods. 'Dried laver' was commercially purchased in a dried form. For 'roasted laver', we roasted the dried laver for 5 sec in an ungreased frying-pan. For 'seasoned laver', we brushed the dried laver with one teaspoon of sesame oil and 0.05 g of table salt and let it rest for 2 h so that the seasoning would be well absorbed. Then, we roasted the seasoned laver for 5 sec in an ungreased frying-pan. Due to the hygroscopic nature of laver, all samples were stored in air-tight plastic bags until analysis.

### Extraction of laver

We used food-grade solvents such as water and different percentages of aqueous ethanol to alleviate safety concerns regarding the use of organic solvent extracts in food. In a preliminary study, we found that water and 70% ethanol were good extraction solvents based on extraction yield and antioxidant activity. Thus, we used water and 70% ethanol as extraction solvents for further study. For each extraction method, samples were cut into small pieces, weighed (~20 g), and 20 volumes of extraction solvent were added to the sample. Samples were extracted for 4 h at 37°C or 100°C. The mixture was centrifuged at 5,000 *g* for 20 min, and the upper layer was transferred to a clean tube. Each extraction was performed three times. Solvent fractions were combined and evaporated to dryness in a vacuum evaporator (EYELA 400 series, Tokyo Rikakikai Co., LTD., Tokyo, Japan). The extracts were freeze-dried (IlshinBiobase, Seoul, Korea), milled to a $<1.0$ mm particle size, and kept in air-tight plastic bags at $-20$°C until analysis. All determinations were performed at least in triplicate, and data are reported on a dry weight basis as mean±standard deviation (SD).

### Determination of total phenolic content

The laver extracts (water or 70% ethanol) were dissolved in deionized water (1 mg/mL) and the Folin-Denis method (15) was used to determine total polyphenol content, with some modifications. Briefly, 0.2 mL of extract was mixed with 0.4 mL of 10% 2 N Folin-Ciocalteu's phenol reagent and allowed to react for 3 min at room temperature, after which 0.8 mL of 10% $Na_2CO_3$ solution was added. The mixture was kept in the dark at room temperature for 1 h, and then absorbance was measured at 750 nm with a microplate reader (Spectra MAX M2, Molecular Device, Sunnyvale, CA, USA). The results are expressed as mg gallic acid equivalents (GAE)/g dry weight.

### Determination of total flavonoid content

Total flavonoid content was measured using the method of Woisky and Salatino (16), with slight modifications. Water or 70% ethanol extracts of laver were dissolved in water (1 mg/mL). Then 100 μL of sample was mixed with 500 μL of distilled water and 30 μL of 5% $NaNO_2$ and allowed to react for 6 min at room temperature. The mixture was added to 60 μL of 10% aluminum chloride and allowed to react for 6 min at room temperature. A 200 μL aliquot of 1 M NaOH and 110 μL of distilled water were added. The mixture was incubated at 25°C for 40 min, and then the absorbance was measured at 415 nm with a microplate reader (Spectra MAX M2). Total flavonoid content was calculated as catechin equivalents (CE) using calibration curves prepared with quercetin standard solutions.

### DPPH radical scavenging activity

The DPPH radical scavenging activities of the laver extracts were determined by the method of Cheung et al.

(17), with minor modifications. The hydrogen atom or electron donation abilities of the samples and some pure compounds were measured from a light-purple colored DPPH methanol solution. One milliliter of various concentrations (100~1,000 μg/mL) of each extract in 10% ethanol was added to a 1 mL DPPH radical solution in methanol (final DPPH concentration, 0.2 mM). The mixture was shaken vigorously, allowed to stand for 25 min, and the absorbance of the resulting solution was measured at 515 nm. Percent inhibition of the DPPH free radical was calculated by the following equation:

$$\text{Inhibition (\%)} = 100 \times (A_{control} - A_{sample})/A_{control}$$

where $A_{control}$ is the absorbance of the control reaction (containing all reagents except the test compound) and $A_{sample}$ is the absorbance with the test compound. Ascorbic acid was used as a control.

**ABTS radical scavenging activity**

The ABTS radical scavenging activities of the laver extracts were determined using the method of Re et al. (18), with minor modifications. The ABTS radical cation ($ABTS^{\bullet+}$) was generated by mixing an aqueous solution of ABTS with a solution of potassium persulfate to achieve a final concentration of 7.4 mM $ABTS^{\bullet+}$ and 2.6 mM of potassium persulfate. This solution was kept in the dark at room temperature for 24 h before use. Then the $ABTS^{\bullet+}$ solution was diluted with phosphate buffered saline (pH 7.4) to an absorbance reading of 0.7±0.03 at 732 nm. Stock solutions of various concentrations (100~1,000 μg/mL) of the samples were prepared in 10% ethanol. Appropriate volumes (determined in preliminary experiments) of the samples were transferred to test tubes containing 950 μL of $ABTS^{\bullet+}$ solution. The solutions were mixed and after 10 min, the absorbance of the solution was measured at 732 nm. The percent inhibition (%) was calculated using the following equation:

$$\text{Inhibition (\%)} = 100 \times (A_{control} - A_{sample})/A_{control}$$

where $A_{control}$ is the absorbance of the control reaction (containing all reagents except the test compound) and $A_{sample}$ is the absorbance with the test compound. Ascorbic acid was used as a control.

**Hydroxyl radical scavenging activity**

Hydroxyl radical scavenging activity was determined as described by Chung et al. (19), with a sight modification. This assay is based on the quantification of the degradation product produced when 2-deoxyribose condenses with TBA. The hydroxyl radical was generated by the $Fe^{3+}$-ascorbate-EDTA-$H_2O_2$ system in 570 μL of a mixture containing 100 μL of 1 mM EDTA, 10 μL of 10 mM $FeCl_3$, 100 μL of 10 mM $H_2O_2$, and 360 μL of 10 mM 2-deoxy-D-ribose. One milliliter of various concentrations of sample was mixed with 570 μL of the aforementioned mixture, 330 μL of 50 mM phosphate buffer (pH 7.4), and 100 μL of ascorbic acid, in that order. After incubation for 1 h at 37°C, 1 mL of the reaction mixture was added to 1 mL of 10% TCA and 1 mL of 0.5% TBA. The final mixture was incubated in a boiling water bath at 100°C for 30 min. After cooling, the flocculent precipitate was removed by adding 3 mL of *n*-buthanol and centrifuging at 10,000 *g* for 25 min. The absorbance of the supernatant was measured at 532 nm against an appropriate blank solution (i.e., distilled water). Trolox was used as a positive control.

**Superoxide anion scavenging activity**

The superoxide radical generated in the xanthine/xanthine oxidase system was determined spectrophotometrically using the NBT product as an indicator (20). The reaction mixture was prepared with 50 μL of sample, 0.5 mL of a 1:1 ratio mixture of 0.4 mM xanthine and 0.24 mM NBT, 0.5 mL of 0.049 U/mL xanthine oxidase, and distilled water, to obtain a final volume of 2.0 mL. After incubation at 37°C for 40 min, 2 mL of 69 mM SDS was added to stop the reaction. The absorbance was measured at 560 nm and compared with that of control samples that had been run without xanthine oxidase. Ascorbic acid was used as the positive control. Percent inhibition (%) was calculated using the following equation:

$$\text{Inhibition (\%)} = \{1 - (A_{sample}/A_{control})\} \times 100$$

where $A_{control}$ was the absorbance of the control (blank, without the test compound) and $A_{sample}$ was the absorbance with the test compound.

**Statistical analysis**

Experimental values are reported as mean±standard derivation of the number of experiments indicated. Significance was assessed using ANOVA-tests in SPSS, version 17.0 (SPSS Inc., Chicago, IL, USA). A probability value of $P < 0.05$ was considered significant.

## RESULTS AND DISCUSSION

**Extraction yield**

We used polar solvents, including water and different percentages of aqueous ethanol (50~90%), to determine extraction yield and antioxidant activity. With respect to extraction yield of bioactive compounds from the laver products, we found that water and 70% ethanol were good extraction solvents.

Table 1 shows the extraction yield of differently processed laver under various extraction conditions. There was considerable variation in extraction yield among the differently processed laver and when different extraction methods were used. The highest extraction yield was found in the 100°C water extracts, whereas the lowest yield occurred in the 37°C 70% ethanol extracts for all three laver processing methods (i.e., dried laver, roasted laver, and seasoned laver). The extraction yield of the 100°C water extract of dried laver was higher than that of the 70% ethanol extract. Interestingly, large differences were observed between the water and 70% ethanol extracts. The 100°C water extraction yields of dried laver, roasted laver, and seasoned laver were 41.3%, 40.6%, and 26.4%, respectively, whereas the 37°C 70% ethanol extraction yields of dried laver, roasted laver, and seasoned laver were 17.9%, 20.2%, and 16.0%, respectively. Laver reportedly contains high levels of water-soluble compounds, such as soluble polysaccharides, proteins, and peptides (2-4), which were less susceptible to extraction by 70% ethanol.

The extraction yields from dried laver, roasted laver, and seasoned laver were higher in the 100°C water extraction condition than in the 37°C water extraction condition. The yield of dried laver extract (i.e., without oil and salt added) was higher than that of the roasted laver extract and the seasoned laver extract. The addition of oil and salt is thought to prevent extraction of water soluble compounds, which may explain the particularly low yield of the seasoned laver extracts. Cho et al. (21) reported that the extraction yields of *Enteromorpha prolifera* by crude, *n*-hexane, chloroform, ethyl acetate, and aqueous extractions were 11.8%, 43.6%, 25.9%, 5.5%, and 20.2%, respectively. These considerable differences in extract yields from various seaweeds may be due to species-specific differences and modifications of extraction conditions, such as solvent, temperature, and time (21).

**Table 1.** Extraction yield (%) of differently processed laver under various extraction conditions

| Processing method | Extraction condition | | |
|---|---|---|---|
| | 100°C, water | 37°C, water | 37°C, 70% ethanol |
| Dried laver | 41.3 | 25.5 | 17.9 |
| Roasted laver | 40.6 | 32.1 | 20.2 |
| Seasoned laver | 26.4 | 21.0 | 16.0 |

**Determination of total phenolic and flavonoid contents**

Seaweeds are rich sources of polyphenolic antioxidants such as flavonols, catechins, and phlorotannis (22). The total phenolic contents of the laver extracts are presented in Table 2. Significant differences in total phenolic contents were found among differently processed laver extracts. The total phenolic content of the extracts tested in this study ranged from 10.81 mg GAE/g extract to 32.14 mg GAE/g extract, depending on the extraction solvent and temperature. Water extraction at 100°C yielded a lower phenolic content than water and 70% ethanol extraction at 37°C. In all tested laver products, 70% ethanol was a more efficient solvent than water for the extraction of polyphenolic compounds. The 70% ethanol extracts of dried laver, roasted laver, and seasoned laver contained 30.18 mg GAE/g extract, 32.14 mg GAE/g extract, and 28.60 mg GAE/g extract, respectively.

Koivikko et al. (23) compared the ability of eight extraction solvents with different polarities to extract soluble polyphenols from *Fucus vesiculosus*. They found that 70% aqueous acetone was more efficient at extracting polyphenolic compounds than water for most seaweed species. The solubility of phenolic compounds is generally higher in polar organic solvents than in water. The most effective extraction solvents are typically aqueous mixtures of methanol, ethanol, or acetone (24). It has been postulated that acetone inhibits protein-polyphenol complex formation during extraction (25) or breaks down hydrogen bonds formed between phenolic groups and protein carboxyl groups (26). In contrast, other compounds such as water soluble polysaccharides, protein,

**Table 2.** Comparison of total phenolic (TPC) and total flavonoid contents (TFC)

| Extraction condition | Processing method | TPC (mg GAE/g extract) | TFC (mg CE/g extract) |
|---|---|---|---|
| 100°C, water | Dried laver | 13.50±0.17$^{a}$ | 1.75±0.55$^{b}$ |
| | Roasted laver | 20.06±0.12$^{b}$ | 0.98±0.51$^{a}$ |
| | Seasoned laver | 10.81±0.13$^{a}$ | ND[1)] |
| 37°C, water | Dried laver | 28.72±0.51$^{c}$ | 1.25±0.44$^{b}$ |
| | Roasted laver | 28.61±0.27$^{c}$ | 0.55±0.35$^{a}$ |
| | Seasoned laver | 20.88±0.48$^{b}$ | ND |
| 37°C, 70% ethanol | Dried laver | 30.18±0.41$^{c}$ | ND |
| | Roasted laver | 32.14±0.22$^{c}$ | ND |
| | Seasoned laver | 28.60±0.55$^{c}$ | ND |

Data are mean±standard deviation of triplicate experiments.
$^{a-c}$Means with different superscripts are significantly different at $P<0.05$.
[1)]ND: Not determined.

and organic acids are simultaneously extracted when using water alone as the extraction solvent (27).

The reported phenolic contents vary among seaweed species and extraction solvents. Cho et al. (21) reported that the total phenolic contents of a crude extract and solvent-partitioned fractions of *E. prolifera*, a type of green seaweed, ranged from 46.2 mg GAE/g to 80.4 mg GAE/g. These values were considerably higher than our results. The phenolic content of the ethyl acetate fraction of *Polysiphonia urceolata*, a red alga, is 73.7 mg GAE/g (28), whereas the phenolic content of the ethanol extract of *Papenfussiella kuromo*, a brown seaweed, is only 0.18 mg GAE/g (29). Devi et al. (30) reported that the *in vitro* antioxidant activities of several seaweeds and the total phenol concentrations of methanol and ethyl ether extracts of *Turbinaria conoides* were 1.23 mg GAE/g and 1.19 mg GAE/g, respectively. Several studies have reported that the antioxidant activity of extracts from various types of seaweed may be correlated with the total phenolic content of the extract (30,31).

The total flavonoid contents of the differently processed laver are presented in Table 2. Laver contained very few flavonoids. No flavonoids were detected in the 70% ethanol extracts of dried laver, roasted laver, or seasoned laver. The flavonoid contents of the 100°C water extracts of dried laver and roasted laver were 1.75 mg CE/g and 0.98 mg CE/g, respectively. No flavonoids were detected in seasoned laver under any of the extraction conditions. Our results indicate that laver is not a good source of flavonoids and that the flavonoid concentration of laver decreases with processing (e.g., with roasting or seasoning).

**Determination of antioxidant activity**

The antioxidant properties of laver that had been extracted with different solvents and at different temperatures were determined by DPPH, ABTS, hydroxyl, and superoxide anion radical scavenging assays. Antioxidant potentials of the samples varied with processing method and extraction condition.

DPPH is commonly used as a substrate to evaluate antioxidant activity. The method is based on the reduction of an ethanolic DPPH solution in the presence of a hydrogen donating antioxidant, resulting in the formation of the non-radical form DPPH-H. The DPPH radical scavenging activity of processed laver is shown in Table 3. The DPPH radical scavenging activity of the water and 70% ethanol extracts of dried laver, roasted laver, and seasoned laver products increased in a concentration-dependent manner (100~1,000 μg/mL). Both 37°C and 100°C water extracts had lower DPPH radical scavenging activity than the 37°C 70% ethanol extract. Extraction at high temperature (i.e., 100°C) may destroy some bioactive compounds and decrease the inhibition of DPPH radical scavenging activities. The highest DPPH scavenging capacity was observed in 37°C 70% ethanol extracts. In contrast, both the 37°C and the 100°C water extracts exhibited relatively weak DPPH scavenging capacity. This suggests that compounds with the strongest DPPH radical scavenging capacity in the 70% ethanol extract may be more soluble in a 70% ethanol than in water. The sesame oil in the seasoned laver may have contained several antioxidant compounds that contributed to DPPH radical scavenging activity.

The 37°C 70% ethanol extract of seasoned laver showed a more powerful inhibitory effect on DPPH radical scavenging activity compared to extracts of the dried laver and the roasted laver products. The average inhibition of DPPH radical formation by a 1,000 μg/mL concentration of the 70% ethanol extract of dried laver

**Table 3.** The DPPH radical scavenging activity (%) of differently processed laver extracts

| Extraction condition | Con (mg/mL) | Processing method | | |
|---|---|---|---|---|
| | | Dried laver | Roasted laver | Seasoned laver |
| 100°C, water | 100 | 3.73±0.12$^{b}$ | 1.55±0.35$^{a}$ | 4.26±0.95$^{b}$ |
| | 200 | 4.37±0.10$^{ab}$ | 2.77±0.45$^{a}$ | 6.09±0.91$^{b}$ |
| | 250 | 5.14±0.78$^{ab}$ | 3.07±0.73$^{a}$ | 7.18±0.70$^{b}$ |
| | 500 | 6.67±0.95$^{ab}$ | 5.04±0.41$^{a}$ | 11.16±0.98$^{b}$ |
| | 1,000 | 12.48±0.61$^{a}$ | 12.17±0.80$^{a}$ | 18.73±1.47$^{b}$ |
| 37°C, water | 100 | 1.77±0.14$^{a}$ | 3.61±0.70$^{b}$ | 4.47±1.44$^{b}$ |
| | 200 | 2.07±0.09$^{a}$ | 3.68±0.19$^{ab}$ | 6.01±1.21$^{b}$ |
| | 250 | 2.29±0.20$^{a}$ | 5.15±0.27$^{b}$ | 7.16±0.27$^{b}$ |
| | 500 | 3.89±0.11$^{a}$ | 7.29±0.75$^{b}$ | 10.22±0.14$^{c}$ |
| | 1,000 | 5.62±0.45$^{a}$ | 13.71±0.38$^{b}$ | 20.01±1.23$^{c}$ |
| 37°C, 70% ethanol | 100 | 6.84±0.40$^{ab}$ | 5.50±0.37$^{a}$ | 8.56±0.71$^{b}$ |
| | 200 | 9.31±1.32$^{b}$ | 6.90±0.71$^{a}$ | 13.16±0.05$^{c}$ |
| | 250 | 10.90±0.80$^{ab}$ | 8.19±0.52$^{a}$ | 14.76±0.46$^{b}$ |
| | 500 | 15.42±0.65$^{b}$ | 11.76±0.17$^{a}$ | 24.58±0.80$^{c}$ |
| | 1,000 | 23.05±0.55$^{b}$ | 19.33±0.36$^{a}$ | 35.64±0.73$^{c}$ |

Data are mean±standard deviation of triplicate experiments.
$^{a-c}$Within the same row, values with different superscripted letters are significantly different at $P<0.05$.

was 35.64%, whereas the average inhibition of DPPH radical formation was 20.01% by the 37°C water extract and 18.73% by the 100°C water extract. These results show that seasoned laver has the strongest DPPH scavenging capacity.

Table 4 shows the electron donating ability (%) of laver extracts as determined by the ABTS radical scavenging method. The 37°C water extract had a more powerful ABTS radical scavenging activity than the 100°C water extract. The ABTS radical scavenging activity of water and 70% ethanol extracts increased in a concentration-dependent manner (100~1,000 μg/mL). At a concentration of 1,000 μg/mL, the percent inhibition of ABTS radical scavenging activity by 37°C water extracts of dried laver, roasted laver, and seasoned laver were 26.51%, 19.66%, and 16.58%, respectively. At the same concentration, the percent inhibition of ABTS radical scavenging activity by 100°C water extracts of dried laver, roasted laver, and seasoned laver were slightly lower (15.75%, 8.57%, and 14.66%, respectively). Extraction at a high temperature (i.e., 100°C) may destroy some bioactive compounds, resulting in decreased inhibition of ABTS radical scavenging activity by processed laver products. The 37°C 70% ethanol extract of seasoned laver showed a more powerful inhibitory effect on ABTS radical scavenging activity than other extraction methods and other laver products. At a concentration of 1,000 μg/mL, the average inhibition of ABTS radical formation by the 70% ethanol extract of seasoned laver was 28.62%, whereas the average inhibition of ABTS radical formation by the 37°C and 100°C water extracts was 16.58% and 14.66%, respectively. Again, the elevated ABTS radical scavenging activity in the seasoned laver condition may have been due to presence of antioxidant compounds in the

**Table 4.** The ABTS radical scavenging activity (%) of differently processed laver extracts

| Extraction condition | Con (mg/mL) | Processing method | | |
|---|---|---|---|---|
| | | Dried laver | Roasted laver | Seasoned laver |
| 100°C, water | 100 | 2.58±0.50$^{b}$ | 0.87±0.54$^{a}$ | 2.14±0.46$^{b}$ |
| | 200 | 4.08±0.38$^{b}$ | 1.80±0.38$^{a}$ | 3.68±0.52$^{ab}$ |
| | 250 | 4.74±0.91$^{ab}$ | 3.10±0.93$^{a}$ | 5.01±1.13$^{b}$ |
| | 500 | 8.84±0.51$^{b}$ | 4.97±0.35$^{a}$ | 8.27±0.30$^{b}$ |
| | 1,000 | 15.75±0.49$^{b}$ | 8.57±0.85$^{a}$ | 14.66±0.84$^{b}$ |
| 37°C, water | 100 | 4.07±0.25$^{a}$ | 3.31±0.31$^{a}$ | 2.13±0.29$^{a}$ |
| | 200 | 6.90±0.21$^{b}$ | 5.37±0.44$^{ab}$ | 3.78±0.24$^{a}$ |
| | 250 | 8.90±0.58$^{b}$ | 6.45±0.55$^{ab}$ | 5.15±0.34$^{a}$ |
| | 500 | 15.36±0.31$^{b}$ | 11.31±0.97$^{ab}$ | 9.00±1.14$^{a}$ |
| | 1,000 | 26.51±1.95$^{b}$ | 19.66±0.73$^{ab}$ | 16.58±0.91$^{a}$ |
| 37°C, 70% ethanol | 100 | 1.32±0.32$^{a}$ | 1.92±0.28$^{a}$ | 3.70±0.19$^{b}$ |
| | 200 | 3.12±0.44$^{a}$ | 2.90±0.47$^{a}$ | 7.70±0.22$^{b}$ |
| | 250 | 3.19±0.88$^{a}$ | 3.39±0.32$^{a}$ | 8.34±0.77$^{b}$ |
| | 500 | 7.08±0.44$^{a}$ | 6.04±0.84$^{a}$ | 15.65±0.38$^{b}$ |
| | 1,000 | 13.60±0.68$^{a}$ | 11.40±0.32$^{a}$ | 28.62±0.08$^{b}$ |

Data are mean±standard deviation of triplicate experiments.
$^{a-b}$Within the same row, values with different superscripted letters are significantly different at $P<0.05$.

**Table 5.** The hydroxyl radical scavenging activity (%) of differently processed laver extracts

| Extraction condition | Con (mg/mL) | Processing method | | |
|---|---|---|---|---|
| | | Dried laver | Roasted laver | Seasoned laver |
| 100°C, water | 100 | 1.35±0.45$^{a}$ | 1.97±1.96$^{a}$ | 7.46±3.78$^{b}$ |
| | 200 | 2.49±0.74$^{a}$ | 2.28±0.65$^{a}$ | 12.22±1.66$^{b}$ |
| | 250 | 6.25±1.86$^{a}$ | 4.25±1.93$^{a}$ | 16.64±3.61$^{b}$ |
| | 500 | 8.39±1.12$^{ab}$ | 6.73±0.35$^{a}$ | 23.74±2.58$^{b}$ |
| | 1,000 | 17.78±2.51$^{b}$ | 13.45±1.82$^{b}$ | 28.45±2.92$^{c}$ |
| 37°C, water | 100 | 9.04±1.77$^{b}$ | 3.73±0.47$^{a}$ | 9.04±1.77$^{b}$ |
| | 200 | 12.22±1.66$^{b}$ | 10.82±2.40$^{a}$ | 13.50±3.47$^{b}$ |
| | 250 | 17.11±1.75$^{b}$ | 12.82±2.29$^{a}$ | 17.11±1.75$^{b}$ |
| | 500 | 23.74±2.58$^{a}$ | 25.61±1.60$^{a}$ | 23.12±3.22$^{a}$ |
| | 1,000 | 37.99±2.76$^{a}$ | 37.18±3.17$^{a}$ | 34.15±3.07$^{a}$ |
| 37°C, 70% ethanol | 100 | 7.21±0.61$^{a}$ | 17.04±2.63$^{b}$ | 15.99±1.36$^{b}$ |
| | 200 | 15.39±1.58$^{a}$ | 18.25±1.95$^{ab}$ | 16.90±1.17$^{c}$ |
| | 250 | 24.52±2.54$^{a}$ | 23.68±2.43$^{a}$ | 20.34±2.51$^{a}$ |
| | 500 | 31.78±2.77$^{ab}$ | 29.34±2.75$^{ab}$ | 25.83±2.73$^{a}$ |
| | 1,000 | 41.58±3.01$^{a}$ | 40.32±2.35$^{a}$ | 39.23±3.34$^{a}$ |

Data are mean±standard deviation of triplicate experiments.
$^{a-c}$Within the same row, values with different superscripted letters are significantly different at $P<0.05$.

sesame oil used.

Hydroxyl radicals, which are the most reactive of the oxygen radicals, are produced by hydrogen peroxide *in vivo* and can react with almost all of the substances in the cell, inducing severe cell damage (32). The hydroxyl radical scavenging activity of processed laver is presented in Table 5. The hydroxyl radical scavenging activity of the water and the 70% ethanol extracts of dried laver, roasted laver, and seasoned laver products increased in a concentration-dependent manner (100~1,000 μg/mL). Both 37°C and 100°C water extracts had lower hydroxyl radical scavenging activities than the 37°C 70% ethanol extract. The highest hydroxyl radical scavenging capacity was observed in the 37°C 70% ethanol extracts. The average inhibition of hydroxyl radical formation by a 1,000 μg/mL concentration of the 70% ethanol extract of dried laver was 41.58%, whereas the average inhibition of hydroxyl radical formation by the 37°C and 100°C water extracts was 37.18% and 13.45%, respectively. For all concentrations tested (250~1,000 μg/mL), dried laver had higher hydroxyl radical scavenging activity than roasted laver and seasoned laver. However, these differences were not statistically significant.

Superoxide, a relatively stable radical generated in living systems, is known to be very harmful to cellular components as it is a precursor to more reactive oxidative species (e.g., single oxygen radicals and hydroxyl radicals) (33). As shown in Table 6, the superoxide radical scavenging activity of the water and 70% ethanol extracts of dried laver, roasted laver, and seasoned laver increase in a concentration-dependent manner (100~1,000 μg/mL). The highest superoxide radical scavenging capacity was observed in 37°C 70% ethanol extracts. At a concentration of 1,000 μg/mL, the average inhibition of superoxide radical formation in the ethanolic extract of seasoned laver was 37.42%, whereas the average inhibition of superoxide radical formation in the ethanol extracts of dried laver and roasted laver were 32.68% and 31.53%, respectively.

While the laver portion of seasoned laver contained only small amounts of polyphenols and flavonoids, the seasoned laver product as a whole contained sesame oil, which may have contributed to seasoned laver's antioxidant activity. Previous reports indicate that sesame oil is significantly resistant to oxidative rancidity (34). Sesame and sesame oil contain diverse bioactive compounds, including sesamine, tocopherol, and phytosterols (35). Konsoula et al. (36) reported that various concentrations of sesame oil are effective at slowing oxidative deterioration. The elevated DPPH, ABTS, hydroxyl, and superoxide radical scavenging capacities of seasoned laver were probably due to the presence of sesame oil and were not direct effects of the laver itself. Antioxidant compounds contained in laver can be extracted by solvent extraction; however, the potency of the antioxidant activity of these extracts differs with different extraction conditions (time, temperature, solvent, etc.). The highest radical scavenging activity was observed in the 37°C 70% ethanol extracts of dried laver, roasted laver, and seasoned laver. The overall results indicate that laver contains bioactive compounds, such as polyphenols and flavonoids, which may have a positive effect on health.

## CONCLUSION

The highest extraction yield was found in the 100°C water extracts of dried laver, roasted laver, and seasoned laver. The lowest extraction yield was found in the 37°C

**Table 6.** The superoxide anion scavenging activity (%) of differently processed laver extracts

| Extraction condition | Con (mg/mL) | Processing method | | |
|---|---|---|---|---|
| | | Dried laver | Roasted laver | Seasoned laver |
| 100°C, water | 100 | 3.84±0.07$^{a}$ | 3.86±0.26$^{a}$ | 4.34±0.20$^{a}$ |
| | 200 | 5.15±0.06$^{a}$ | 4.55±0.29$^{a}$ | 6.19±0.75$^{a}$ |
| | 250 | 7.28±0.09$^{a}$ | 6.32±0.13$^{a}$ | 8.44±0.65$^{a}$ |
| | 500 | 15.74±0.08$^{a}$ | 13.51±0.11$^{a}$ | 17.47±0.80$^{ab}$ |
| | 1,000 | 26.26±0.06$^{a}$ | 23.13±0.03$^{a}$ | 28.84±0.09$^{ab}$ |
| 37°C, water | 100 | 3.42±0.27$^{a}$ | 3.53±0.08$^{a}$ | 4.08±0.21$^{a}$ |
| | 200 | 4.46±0.42$^{ab}$ | 4.77±0.08$^{a}$ | 5.83±0.12$^{a}$ |
| | 250 | 7.43±1.62$^{a}$ | 8.93±0.05$^{a}$ | 7.22±0.07$^{a}$ |
| | 500 | 12.48±3.65$^{a}$ | 14.77±0.12$^{a}$ | 12.28±0.08$^{a}$ |
| | 1,000 | 23.14±0.03$^{c}$ | 23.55±0.03$^{a}$ | 23.05±0.28$^{a}$ |
| 37°C, 70% ethanol | 100 | 5.67±0.32$^{a}$ | 5.52±0.18$^{a}$ | 6.36±0.28$^{a}$ |
| | 200 | 8.67±0.30$^{a}$ | 8.18±0.07$^{a}$ | 10.66±0.04$^{b}$ |
| | 250 | 10.13±0.46$^{a}$ | 10.61±0.13$^{a}$ | 13.31±0.05$^{b}$ |
| | 500 | 18.88±0.21$^{a}$ | 18.27±0.11$^{a}$ | 21.45±0.12$^{b}$ |
| | 1,000 | 32.68±0.13$^{a}$ | 31.53±0.06$^{a}$ | 37.42±0.10$^{ab}$ |

Data are mean±standard deviation of triplicate experiments.
$^{a-c}$Within the same row, values with different superscripted letters are significantly different at $P<0.05$.

70% ethanol extracts of dried laver, roasted laver, and seasoned laver. The polyphenol contents of the extracts varied with processing method; total polyphenol contents ranged from 10.81~32.14 mg GAE/g extract, depending upon the extraction solvent and the extraction temperature. Across all tested laver products, 70% ethanol was more efficient at extracting polyphenolic compounds than water. Laver contained very few flavonoids, and no flavonoids were detected in the 70% ethanol extracts of dried laver, roasted laver, or seasoned laver. The highest DPPH scavenging capacity was observed in 37°C 70% ethanol extracts. In contrast, both 37°C and 100°C water extracts exhibited relatively weak DPPH scavenging capacities. The 37°C 70% ethanol extract of seasoned laver had more powerful inhibitory effects for ABTS radical scavenging than other extraction methods across all laver products. In addition, 37°C 70% ethanol extracts had the highest superoxide radical scavenging capacities and hydroxyl radical scavenging activities.

## AUTHOR DISCLOSURE STATEMENT

The authors declare no conflict of interest.

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