

Evaluation of Phytochemical Composition and Antioxidant Capacity in Various Leafy Vegetables

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Abstract

Current study investigated phytochemical compositions and antioxidant capacity of Korean leafy vegetables including chajogi, gomchwi, meowi and sseumbagwi. β -carotene, total soluble polyphenol, total flavonoids contents were determined, and antioxidant capacity were evaluated by various methods. β -carotene, total soluble polyphenol, total flavonoids contents in gomchwi were significantly higher (as much as 19 folds) compare to other vegetables ($p < 0.05$). Sseumbagwi extract (100 $\mu\text{g/mL}$) was removed 78.6% of superoxide radicals in xanthin-xanthin oxidase system measured by EPR. Gomchwi showed the highest nitrate scavenging activity as 94.3% at pH 1.2. In SOD-like activity, chajogi, gomchwi, and meowi were evaluated in the range of 12.6~24.5%. All samples were revealed to prevent the reaction of ferrous chloride during 9 days, which were comparable ability with 125 mg/100 g of α -tocopherol. The rich phytochemical contents of gomchwi and sseumbagwi influenced to high antioxidant capacity.

Key words: leafy vegetables, antioxidants, total soluble phenolics, total flavonoids, EPR

INTRODUCTION

Interests in phytochemicals from various fruits and vegetables have increased in recent years, due to positive associations between consumption and resulting health benefits. Several studies have shown that polyphenolics in fruits and vegetables can directly or indirectly influence human health through properties such as anti-carcinogenicity (1,2), radical scavenging activity (3,4), skin damage prevention (5,6), anti-aging (7), as well as effects against neurodegenerative diseases such as Alzheimer's and Parkinson's diseases (8,9). Accordingly, the traditional Korean diet has gained attention since it is based on various vegetables and herbs, including chajogi (*Perilla frutescens* var. *acuta*), gomchwi (*Ligularia fischeri*), meowi (*Petasites japonicus*), and sseumbagwi (*Ixeris dentata*). These leafy vegetables are consumed as foods and are also traditionally utilized as medicinal herbs due to their specific activities for depression (10), infection (11), inflammation (12,13), and allergies (14). Various trials have been conducted to evaluate the phytochemical compositions of leafy vegetables, where quercetin, catechin, and epicatechin were reported as the main active compounds (15-18). However, a limited

amount of information is available on their phytochemical contents relating to antioxidant capacity. Therefore, the objective of this study was to evaluate the phytochemical contents of traditional leafy vegetables, including chajogi, gomchwi, meowi, and sseumbagwi, in relation to the overall assessment of their antioxidant properties. The information from this study helps to reveal the value of consuming a traditional Korean diet, and shows the possibility of utilizing leafy vegetables for various functional food applications.

MATERIALS AND METHODS

Vegetables and chemicals

The chajogi (*Perilla frutescens* var. *acuta* KUDO), gomchwi (*Ligularia fischeri* (Ledeb.) TURCZ), meowi (*Petasites japonicus* (S.et Z.) MAXIM), and sseumbagwi (*Ixeris dentata* (Thunb.) NAKAI) for this study were purchased at local markets in the dried forms. Ammonium thiocyanate, ferrous chloride, ferulic acid, Folin-Ciocalteu's reagent, EDTA (ethylenediaminetetraacetic acid), gallic acid, linoleic acid, pyrogallol, quercetin, sodium nitrite, sodium phosphate monobasic, tris[hydroxymethyl] amino methane, aluminum nitrate, potassium

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acetate, 1-naphthylamine, sodium citrate tribasic dihydrate, sodium phosphate dibasic heptahydrate, sulfanilic acid (Sigma-Aldrich Chemical Co., Steinheim, Germany), hydrocholic acid (Duksan Pharmaceutical, Kyungkido, Korea), and α -tocopherol (Kato Chemical, Tokyo, Japan) were purchased as analysis grade.

Chemical analyses of antioxidant compounds

Chemical analyses were conducted to characterize the major phytochemicals in the leafy vegetables and to evaluate their antioxidant properties. Each sample (100 g) was blended for 2 minutes, and kept in air-tight container at -15°C until further use. Phytochemicals were extracted three times with 70% ethanol (100 mL) for 2 hrs in mechanical shaker at room temperature. Each extract was filtered through Whatman No. 1 filter paper following solvent evaporation until dryness, and residues redissolved in 10% ethanol.

Total soluble phenolics were determined using the Folin-Ciocalteu assay (19) and were expressed as ferulic acid equivalents (FAE, mg/100 g). Total flavonoids were assessed by absorbance at 415 nm after 40 min of reaction with 10-fold diluted extracts (0.5 mL), 10% aluminum nitrate (0.1 mL), 1 M potassium acetate (0.1 mL), and 70% ethanol (4.3 mL) (20). Data were expressed as quercetin equivalents (mg/100 g). For β -carotene analysis, dried samples (1 g) were extracted twice with methanol and acetone: hexane (1:1 v/v), and the combined solvents were removed under pressure (30°C). The concentrated extracts were partitioned with 30 mL of hexane and washed with distilled water three times. The saponification was conducted under nitrogen atmosphere adding supersaturated KOH/MeOH at room temperature for 30 min. Subsequently, the samples were washed with distilled water three times, dried over sodium sulphate, adjusted to 100 mL with hexane, and then absorbance was measured at 448 nm. β -carotene was used as a standard for the calibration curve (21).

EPR analysis

Superoxide anion radical and hydroxyl radical scavenging activities of samples were evaluated by an electron paramagnetic resonance (EPR) spectrometer (JEOL-JES-TE5200, Japan). Ethanolic samples were prepared in phosphate buffer at various concentrations of 20, 50, and 100 $\mu\text{g}/\text{mL}$. Superoxide anion radical scavenging activity was determined by a xanthin-xanthin oxidase superoxide anion generating system. Eight microliters of xanthine (1 mM) were placed in a test tube, and 185 μL of potassium phosphate buffer (0.1 M, pH 7.4) containing the samples, 2 μL of diethylene triamine pentaacetic acid (DTPA, 50 mM), 2 μL of 5, 5-dimethyl-1-

pyrroline N-oxide (DMPO, 180 mM), 1 μL of catalase (1 μM), and 2 μL of xanthin oxidase were added successively (22,23). Hydroxyl radical scavenging activity was measured with a Fenton reaction system containing 4 μL of ethylenediaminetetraacetic acid (EDTA, 200 μM), 2 μL of DMPO (90 mM), 4 μL of FeSO_4 (200 μM), and 5 μL of H_2O_2 (1 μM) in 185 μL of potassium phosphate buffer (0.1 M, pH 7.4), which already contained the samples (24). EPR measurements were made after 2 min at 20°C . Radical scavenging activity was determined by measuring the reduction in the heights of peaks for either superoxide anion or hydroxyl radical of samples to those of the control, and was calculated as follows: radical scavenging activity (%) = $[100 - (A_1/A_0) \times 100]$, where A_0 was the control peak height and A_1 was the sample peak height.

Nitrite scavenging activity

Nitrite scavenging activity was measured by the colorimetry method, modified from Kato et al. (25) and Lim et al. (26), using Griess reagent. The ethanol extracts (1 mL) were added to 2 mL of NaNO_2 (1 mM) and pH was adjusted to 1.2, 3.0, and 6.0 with 0.1N HCl and 0.2 M citric acid. Each mixture was made up to 10 mL with buffers and kept at 37°C for 1 hr. Then, 1 mL of the mixture was reacted with 2 mL of 2% acetic acid and 0.4 mL of Griess reagent for 15 min at room temperature. The color intensity was measured by a spectrophotometer at 520 nm, and activity was expressed as nitrite scavenging activity (%) = $100 - [(A_2 - A_1)/A_0] \times 100$, where A_0 was the absorbance of the control, A_1 was the absorbance of the sample without Griess reagent, and A_2 was the absorbance of the sample with Griess reagent.

Superoxide dismutase (SOD)-like activity

SOD-like activity was determined by measuring the ability of samples to inhibit pyrogallol autoxidation, as similar to superoxide dismutase, according to the modified method of Marklund and Marklund (27). The samples (0.2 mL) were added to 0.2 mL of pyrogallol (7.2 mM) and 3 mL of Tris-HCl buffer (50 mM, pH 8.5), which contained 10 mM EDTA. Reaction was stopped by adding 1 mL of HCl (1 N) after 10 min (25°C) and the absorbance was measured at 420 nm. SOD-like activity (%) was expressed as follows: SOD-like activity (%) = $100 - [(A_2 - A_1)/A_0] \times 100$, where A_0 was the absorbance of the control, A_1 was the absorbance of the sample without reagent, and A_2 was the absorbance of the sample with reagent.

Ferric thiocyanate (FTC) method

The FTC method was adapted from Osawa and

Namiki (28). The samples (4 mg or 4 mL) in 99.5% ethanol were mixed with 2.51% linoleic acid in 99.5% ethanol (4.1 mL), 0.05 M phosphate buffer (pH 7.0, 8 mL), and distilled water (3.9 mL). The mixtures were kept in screw cap containers under dark conditions at 40°C. Then, 9.7 mL of 75% ethanol and 0.1 mL of 30% ammonium thiocyanate were added to 0.1 mL of this solution. Precisely 3 min after adding 0.1 mL of 2×10^{-2} M ferrous chloride in 3.5% hydrochloric acid to the reaction mixture, the absorbance of the resulting red color was measured at 500 nm every 24 hrs, up until one day after the absorbance of the control reached maximum. The control and standard were subjected to the same procedure as the samples except either water or 4 mg of α -tocopherol were added instead of sample.

Statistical analysis

Data represent the means of duplicate analyses. Analysis of variance and Pearson's correlation tests were conducted using JMP5 software (29); mean separation was conducted using the LSD test ($p < 0.05$).

RESULTS AND DISCUSSION

Phytochemical composition

Contents of carotenoids, total soluble phenolics, and total flavonoids were determined in the leafy vegetables, which included chajogi, gomchwi, meowi, and sseumbagwi. Overall, gomchwi and sseumbagwi contained higher concentrations of the examined phytochemicals than chajogi and meowi (Table 1). The β -carotene contents of the dried gomchwi and sseumbagwi were determined as 157.4 mg/100 g and 116.2 mg/100 g, respectively. Considering that vegetables commonly contain over 90 ~95% moistures, fresh gomchwi and sseumbagwi might contain 78.7 ~157.4 mg/kg of β -carotene, which are fairly high concentrations for vegetables. Spinach is known to be a rich source of carotenoids, and concentrations ranging from 87 to 119 mg/kg (FW) have been reported, which were dependent on storage and processing con-

ditions (30). The total soluble phenolics of samples were evaluated using Folin-Ciocalteu reagent and were quantified as ferulic acid equivalents. The assessments revealed that each prepared sample contained high levels of total soluble phenolics, in the order of gomchwi (3,782.6 mg/100 g), sseumbagwi (2,303.5 mg/100 g), chajogi (1,459 mg/100 g), and meowi (308.1 mg/100 g). A similar pattern was found for total flavonoid content, which ranged from 163.0 to 3,223 mg/100 g. Positive correlation ($R^2=0.97$) was found between the total soluble phenolics and total flavonoids contents. Additionally, anthocyanin compounds were only found in the chajogi with 42.87 mg/100 g (data not shown), and another study reported that anthocyanins were distributed in the leaf and stalk in cyanidin form (31).

Scavenging activity for superoxide anion and hydroxyl radicals by EPR

To determine the reactive oxygen species scavenging ability of the leafy vegetables, superoxide anion and hydroxyl radicals were generated from a xanthin-xanthin oxidase system and Fenton system, respectively. Due to the weak stabilities of the radicals, DMPO was added to trap the radicals, and changes in the intensity of DMPO adduct (DMPO-OOH or DMPO-OH) signals were evaluated by EPR (24). Each sample showed efficiency for removing superoxide radicals at various concentrations (Fig. 1), for examples, chajogi, gomchwi, and sseumbagwi had superoxide anion scavenging abilities greater than 40.4%, which was the capacity for 10 μ g/mL of quercetin to remove radicals. In the concentration range of 20 ~100 μ g/mL, the samples exhibited dose-dependent scavenging activities against superoxide radicals; however, gomchwi did not show a significant difference between the concentrations of 50 and 100 μ g/mL. Among the tested samples, the sseumbagwi extract showed the highest overall scavenging activity at the 100 μ g/mL. The high superoxide scavenging activity of sseumbagwi seemed to be the result of high levels of phytochemical contents, due to outcome of Pearson's correlation analysis as 0.89 and 0.79 at the concentrations of 50 μ g/mL of sample with total soluble phenolics and total flavonoid contents, respectively. Comparing to their superoxide radical scavenging activities, the leafy vegetables in this study revealed lower capacities for removing hydroxyl radicals. Chajogi was the only sample to show activity, with 4.89% at 1000 μ g/mL, whereas none of the other vegetables showed hydroxyl radical scavenging activities (Table 2). Leafy vegetables such as chajogi and gomchwi (32,33) have been evaluated for their radical scavenging abilities for DPPH radicals, hydroxyl radicals, and alkyl radicals, and

Table 1. Concentrations of β -carotene, total soluble phenolics, and total flavonoids on leafy vegetables

	β -carotene (mg/100 g) ¹⁾	Total soluble phenolic (mg/100 g) ²⁾	Total flavonoid (mg/100 g) ³⁾
Chajogi	32.0 \pm 1.4 ^{c4)}	1459 \pm 28.4 ^c	859 \pm 15.3 ^c
Gomchwi	157.4 \pm 8.5 ^a	3782 \pm 14.5 ^a	3223 \pm 70.0 ^a
Meowi	13.7 \pm 0.8 ^d	308.1 \pm 3.2 ^d	163 \pm 8.0 ^d
Sseumbagwi	116.2 \pm 4.8 ^b	2303 \pm 10.0 ^b	1305 \pm 53.9 ^b

¹⁾Expressed in β -carotene equivalent.

²⁾Expressed in ferulic acid equivalent.

³⁾Expressed in quercetin equivalent.

⁴⁾Values within columns with similar letters not significantly different for each value (LSD test, $p < 0.05$).

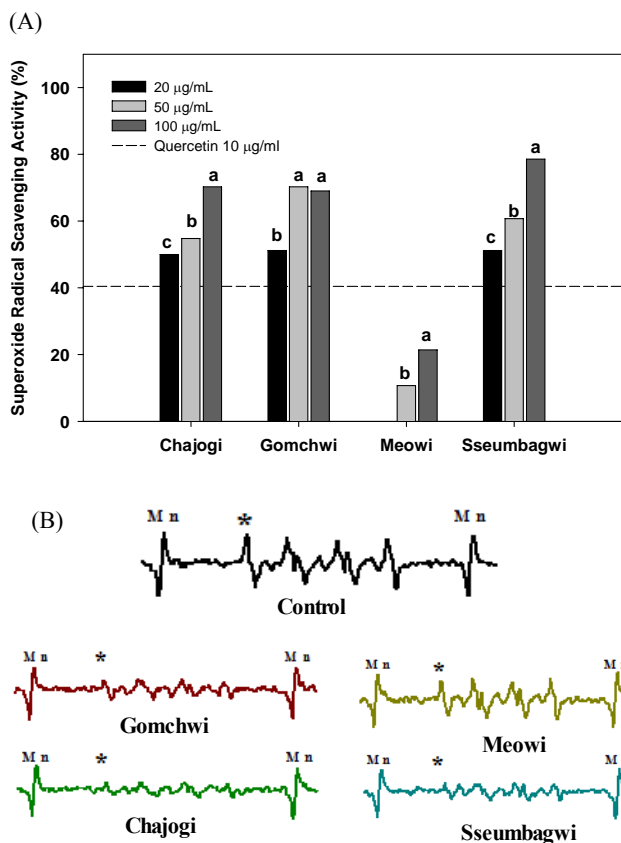


Fig. 1. Superoxide radicals scavenging activity (%) on ethanolic extracts of leafy vegetables at various concentrations (20, 50, 100 µg/mL) comparing quercetin standard (10 µg/mL) (A) and according electron paramagnetic resonance (EPR) spectra at 50 µg/mL. Same letters within samples are not significantly different (LSD test, $p < 0.05$). The conditions of EPR analysis: Microwave frequency 9.42 [GHz], microwave power 5 [mW], modulation amplitude 0.1 [mT]. Asterisks (*) indicate the peaks of DMPO-OOH for measuring.

Table 2. Hydroxyl radicals scavenging activity (%) measured by electron paramagnetic resonance (EPR) spectrometer on quercetin standard and ethanolic extracts of various leafy vegetables

Sample	Concentrations (µg/mL)	OH [•] scavenging activity (% of control)
Chajogi	1000	4.89
Gomchwi	1000	None
Meowi	1000	None
Sseumbagwi	1000	None
Quercetin	50	5.80

are suggested to be useful as natural antioxidant sources and food supplements.

Nitrite scavenging activity

Nitrite scavenging activity was measured by the capacity of samples to remove nitrosamine, which is known to increase the risk of cancer by converting to diazoalkane, proteins and intracellular components (34, 35). In general, nitrosamines are produced from nitrites

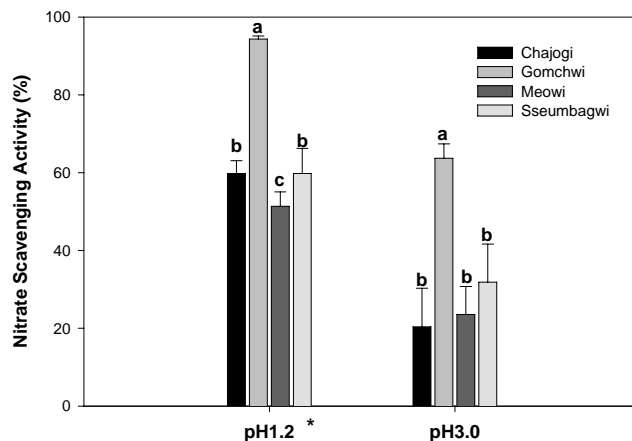


Fig. 2. Nitrite scavenging activity (%) on ethanolic extracts leafy vegetables at pH 1.2 and pH 3.0. Values are expressed as the mean ± SD (n=3). Same letters within pH are not significantly different (LSD test, $p < 0.05$). Asterisks (*) indicate significantly different between pHs (LSD test, $p < 0.05$).

and secondary amines, which often occur in proteins under acidic conditions such as in the stomach. One way to inhibit nitrosamine formation is to degrade nitrite itself or to remove secondary amines by chemicals having reducing abilities including polyphenolic compounds (25). Nitrate scavenging activities of the prepared vegetable samples were assessed under pH 1.2 and 3.0 (Fig. 2). Gomchwi showed the highest activity as 94.3%, and the others also had good activities, ranging from 51.4 to 59.8% at pH 1.2. The elevation of pH decreased the activities of the samples to an average of 31.6%. Under the higher pH, however, gomchwi still maintained a scavenging activity of 63.4%, which can be comparable to commercial antioxidants such as vitamin E or BHA (100 µg/mL) as reported by Lee et al. (35). The flavonoid contents of the vegetable samples seemed to significantly relate to their nitrate-removing abilities, having Pearson correlation coefficients of 0.96 and 0.89 at pH 1.2 and pH 3.0, respectively. Research by Kim et al. (36) has supported the nitrite scavenging ability of flavonoids in natural plants by showing that NO production was inhibited by the reduction of inducible nitric oxide synthase (iNOS) expression.

SOD-like activity

Superoxide dismutase (SOD) is one of the most important factors in oxidative defense systems by catalyzing superoxide into oxygen and hydrogen peroxide. SOD-like activity is principally measured by the extent of inhibition on the automatic oxidation reaction of pyrogallol, which is reactive with superoxide and displays browning material. As shown in Fig. 3, chajogi, gomchwi, and meowi showed SOD-like activities at 12.6%, 24.5%, and 23.8%, respectively. The SOD-like activities

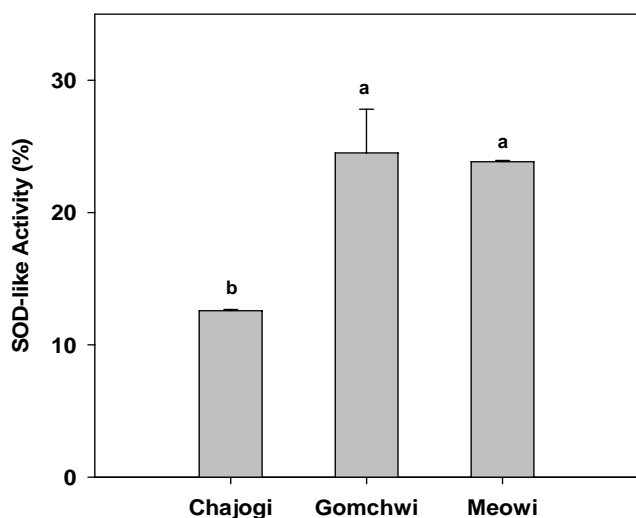


Fig. 3. SOD-like activity (%) on ethanolic extracts of leafy vegetables. Values are expressed as the mean \pm SD (n=3). Same letters within pH are not significantly different (LSD test, $p < 0.05$).

of the tested vegetables were similar to those of kale and radish, which were reported as 26.7% and 24.1%, respectively (37). Fruits and vegetables are known to efficiently protect pyrogallol from oxidation due to the abundant antioxidants they contain, including polyphenol compounds.

Antioxidant activity in the linoleic acid peroxidation system

The ferric thiocyanate (FTC) method was applied to determine the antioxidant activity of samples by measuring the peroxide level during the initial stage of lipid oxidation. As shown in Fig. 4, all samples prevented the reaction of ferrous chloride over 9 days, and had abilities comparable to 125 mg/100 g of α -tocopherol.

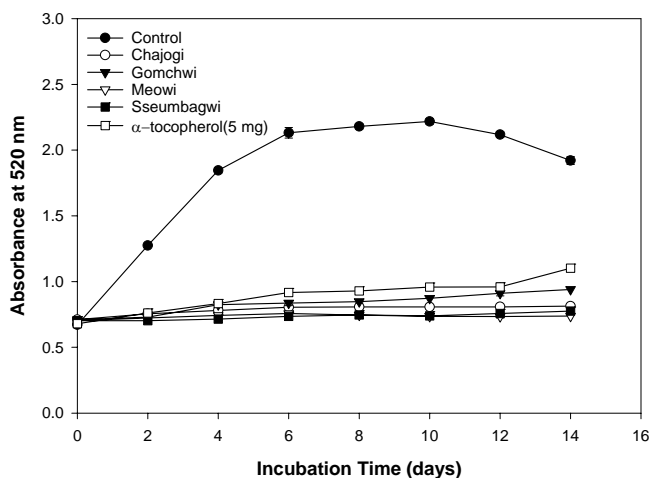


Fig. 4. Antioxidant activity as measured by the FTC method on ethanolic extracts of leafy vegetables. Absorbance values represent means of triplicates from different samples.

High concentrations of total soluble phenolics or total flavonoids probably play a key role to retard lipid oxidation, as previously discussed for the antioxidant activity of gomchwi by FTC and TBA (thiobarbituric acid) methods (32).

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