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RESEARCH NOTE

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Changes in Antioxidant Activity of *Rehmannia radix* Libosch with Heat Treatment

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Abstract This study evaluated the effects of heat treatment on antioxidant activity of *Rehmannia radix* Libosch (RRL). RRL was heated at various temperatures (110-150°C) for various times (1-5 hr), and the total polyphenol, flavonoid content, and antioxidant activity were investigated. With increased heating temperature and exposure time, total content of polyphenol, flavonoid, as well as antioxidant activity increased. The highest total polyphenol and flavonoid contents were 21.65 and 3.56 mg/g, respectively, these values were occurred after heating for 3 hr at 150°C (RRL was 5.09 and 0.83 mg/g, respectively). The 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activity was highest value of 83.46% after heating for 3 hr at 150°C. The 2,2-azino-bis-3-ethylbenzothiazoline-6-sulfonic acid (ABTS) cation radical scavenging activity was highest value of 20.43 mg ascorbic acid (AA) eq/g after heating for 2 hr at 150°C. There were highly significant differences in the total polyphenol, flavonoid content, and antioxidant activity among heating temperatures and times ($p<0.001$), with heating temperature having the greater effect.

Key words: *Rehmannia radix* Libosch, heat treatment, antioxidant activity, polyphenol, flavonoid

Introduction

Rehmannia radix Libosch is a perennial medicinal plant that originates from China and belongs to the Scrophulariaceae. For centuries, many medical books in China, Japan, and Korea listed this plant's roots for medicinal purposes (1). Although there are 7 species of *Rehmannia*, only 3 are used in medicine: *R. radix* Libosch in Korea, *R. radix* var. *purpurea* Makino in Japan, and *R. radix* var. *hueichingensis* (Chao and Schih) Hsiao in China. *R. radix* is generally cultivated in the southern provinces of Korea because of the mild climate and is used in three ways: fresh, dried, and steamed. Each of these forms has different medicinal effects (2).

R. radix contains rehmaglutin A, B, C, and D as iridoids, and catalpol, aucubin, leonuride, melittoside, rehmanniosides, glucosides, amino acids, and stigmasterol as iridoids with glucosides, and has been reported to have medical effects such as the reduction of blood pressure in rabbits, diuretic properties in mice with glycosuria, and antibiotic effects against bacteria (3). *R. radix* also contains resveratrol, phenolic compounds, free amino acids, and superoxide dismutase (SOD), all of which have been reported to be physiologically active substances that have anti-oxidative properties, aid the defense mechanisms, or are chemicals related to resistance to environmental stresses in humans or plants (4,5).

Browning due to heat treatment is the result of several reactions. These include Maillard condensation between reducing sugars and amino acids, caramelization, and ascorbic acid browning processes (6). Some studies have examined the chemical and physical properties of foods in response to high temperature and pressure treatment (HTPT). Recent studies have shown that thermally processed foods, especially fruits and vegetables, have higher biological activity because of chemical changes during heat treatment (7,8). For example, polyphenol content, flavonoid content, and antioxidant activity increase with an increase in HTPT in plants such as ginseng (9), licorice (10), garlic (11,12), onion (13), pear (14), and *shiitake* mushroom (15).

Thus, to investigate the effects of heat treatment on antioxidant activity of *R. radix* Libosch (RRL), RRL was heated at various temperatures (110-150°C) for various times (1-5 hr), and the total polyphenol, flavonoid content, and antioxidant activity were investigated.

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Materials and Methods

Sample preparation *R. radix* Libosch (RRL; dried sample) was supplied by the Sewon Pharmacy Co. (Namyangju, Korea), harvested in 2005 and stored at −20°C. *R. radix* Libosch (RRL) was placed in a sample bottle and sealed tightly. Sample bottles were heat-treated using high-pressure steam generated by temperature and steam pressure controlling apparatus (Jisico, Seoul, Korea). Samples were heated to temperatures of 110, 120, 130, 140, or 150°C for 1, 2, 3, 4, or 5 hr (9-14). Heated samples were extracted by reflux extraction with water at 95°C for 2 hr and centrifuged at 1,200×g for 10 min. The supernatant was filtered through a 0.45-μm syringe filter (Millipore, Billerica, MA, USA). The extract was kept at −20°C until analysis. *R. radix* Preparata (RRP; Sewon Pharmacy Co.) processed with traditional method (9 times steaming) on RRL was used as positive control.

Total polyphenol contents The total polyphenol content

of heated RRL extracts was determined using the Folin-Ciocalteu method (15). In a 10-mL test tube, 2 mL of 2% Na_2CO_3, 0.1 mL of extract appropriately diluted, and 0.1 mL of 50% Folin-Ciocalteu phenol reagent (Sigma-Aldrich, St. Louis, MO, USA) were added and mixed. After exactly 30 min, the absorbance was read at 750 nm, and the total polyphenol concentration was calculated from a calibration curve (R^2=0.9969) that was obtained using tannic acid (Sigma-Aldrich) as a standard (20-200 μg/mL). All extracts were analyzed in triplicate.

Total flavonoid contents The total flavonoid content of heated RRL extracts was determined using the colorimetric method described by Choi *et al.* (15), with some modifications. A standard solution or extract (250 μL) was mixed with 1.25 mL of distilled water and 75 μL of 5% $NaNO_2$. After 5 min, 150 μL of 10% $AlCl_3 \cdot 6H_2O$ was added. After 6 min, 500 μL of 1 M NaOH and 275 μL of distilled water were added to the mixture. The solution was mixed well, the absorbance was read at 510 nm, and the total flavonoid concentration was calculated from a calibration curve (R^2=0.9991) that was obtained using (+)-catechin hydrate (Sigma-Aldrich) as a standard (20-200 μg/mL). All extracts were analyzed in triplicate.

DPPH radical scavenging activity The scavenging activity of heated RRL extracts on the 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical was measured according to the methods of Tepe *et al.* (16), with some modifications. An aliquot of 0.8 mL of 0.2 mM DPPH (Sigma-Aldrich) methanolic solution was mixed with 0.2 mL of extract. The mixture was shaken vigorously and left to stand for 10 min under low light. The absorbance was measured at 520 nm. The DPPH radical scavenging activity (electron donating activity, EDA, %) was calculated as $(1-A_{sample}/A_{control})\times 100$, where A_{sample} is the absorbance in the presence of sample and $A_{control}$ is the absorbance in the absence of sample. All extracts were analyzed in triplicate.

Total antioxidant activity The total antioxidant activity of heated RRL extracts on the 2,2-azino-bis-3-ethylbenzothiazoline-6-sulfonic acid (ABTS) cation radical was measured according to the methods of Re *et al.* (17) and Hwang *et al.* (18), with some modifications. The ABTS (Sigma-Aldrich) cation radical was generated by adding 7 mM ABTS to 2.45 mM potassium persulfate (Sigma-Aldrich) solution, and the mixture was left to stand overnight in the dark at room temperature. The ABTS cation radical solution was diluted with distilled water to obtain an absorbance of 1.4-1.5 at 735 nm (molar extinction coefficient, $e=3.6\times10^4$/mol/cm; 19). Diluted ABTS cation radical solution (1 mL) was added to 50 μL of extract, ascorbic acid standard solution, or distilled water. After 60 min, the absorbance was measured at 735 nm using a spectrophotometer (UV-1650PC; Shimadzu, Kyoto, Japan). The ABTS cation radical scavenging activity was expressed in terms of ascorbic acid equivalent antioxidant capacity (AEAC) as mg of ascorbic acid equivalents (AA eq)/g of sample (15). The AEAC was calculated as $AEAC=(\Delta A_{sample}/\Delta A_{aa})\times C_{aa}\times V\times(100/W_{sample})$, where ΔA_{sample} is the change in absorbance in the presence of extract, ΔA_{aa} is the change in absorbance after the addition of ascorbic acid standard solution, C_{aa} is the concentration of the ascorbic acid standard solution (mg/mL), V is the volume of extract (mL), and W_{sample} is the weight of sample used for extraction (g). All extracts were analyzed in triplicate.

Statistical analysis The results were reported as mean± standard deviation (SD). The significance of differences among treatment means was determined using one-way analysis of variance (ANOVA), using SAS version 9.1 (SAS Institute, Cary, NC, USA), with a significance level of 0.05. Correlations from regression analysis between the parameters were also determined.

Results and Discussion

Total polyphenol contents Phenolic compounds are secondary metabolic products that occur throughout the plant kingdom. They contain the phenolic hydroxyl group, which has an antioxidant effect through interactions with the phenol ring and its resonance stabilization effect (16, 20,21). There were highly significant differences in the total polyphenol contents of heated RRL among the heating temperatures and times ($p<0.001$; Fig. 1), with heating temperature having the greater effect (Table 1). The total polyphenol content of heated RRL increased significantly with increased heating temperature (110 to 150°C) and time (1 to 5 hr; $p<0.001$). The total polyphenol content in RRL and RRP extracts was 5.09 and 13.90 mg/g, respectively. The total polyphenol content of heated RRL increased gradually when heated at 110 to 150°C for 3 hr and then decreased with longer heating times. Heating at 150°C for 3 hr increased the total polyphenol content of heated RRL to 21.65 mg/g. The total polyphenol contents of heated RRL increased significantly with increasing temperature and time. Our results are consistent with those of previous studies. For example, Dewanto *et al.* (22) demonstrated that there is a significant increase in soluble phenolic compounds in sweet corn after heating. Also, Woo *et al.* (13) reported that total polyphenol content in onion was increased with increased heating temperatures and exposure times. Heating releases bound polyphenolic compounds through the

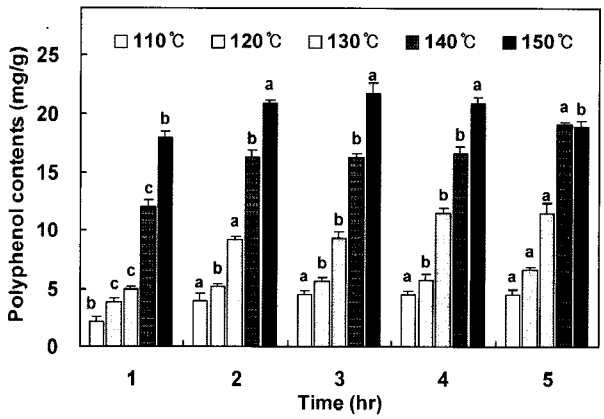

Fig. 1. Change of total polyphenol content of heated *Rehmannia radix* Libosch extract. *R. radix* Libosch (control), 5.09±0.35 mg/g; *R. radix* Preparata (positive control), 13.90±1.38 mg/g. Values represent the mean±SD of 3 replications. Any means in the same column followed by the same letter are not significantly ($p<0.05$) different by Duncan's multiple-range test.

Table 1. Analysis of variance for total polyphenol, flavonoid content, electron donating ability (EDA), and ascorbic acid equivalent antioxidant capacity (AEAC) of heated *Rehmannia radix* Libosch extract

	Variable[1)]	DF	Sum of squares	F-value
Polyphenol	X_1	4	2,881.72	606.69***[2)]
	X_2	4	151.71	31.94***
Flavonoid	X_1	4	62.94	200.38***
	X_2	4	5.97	19.02***
EDA	X_1	4	39,167.25	285.75***
	X_2	4	2,609.89	19.04***
AEAC	X_1	4	1,031.98	448.48***
	X_2	4	187.13	81.32***

[1)] X_1, Heating temperature (°C); X_2, Heating time (hr)
[2)] ***$p<0.001$.

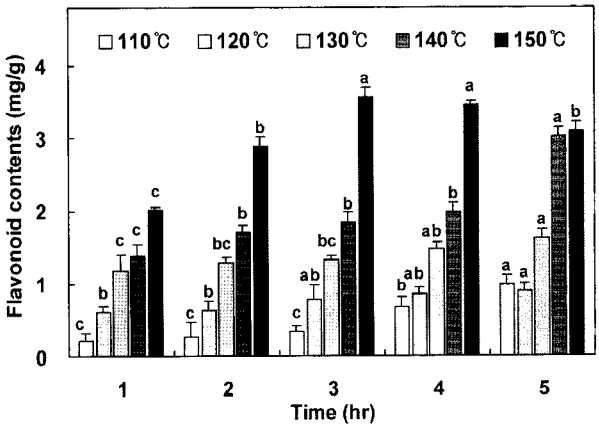

Fig. 2. Change of total flavonoid content of heated *Rehmannia radix* Libosch extract. *R. radix* Libosch (control), 0.83±0.20 mg/g; *R. radix* Preparata (positive control), 1.07±0.06 mg/g. Values represent the mean±SD of 3 replications. Any means in the same column followed by the different letters are significantly different ($p<0.05$) by Duncan's multiple-range test.

breakdown of cellular constituents (15). Jeong *et al.* (23) showed that polyphenolic compounds in citrus peels are liberated by heating.

Total flavonoid contents The total flavonoid content was affected by heating temperature and time in a manner similar to the total polyphenol content (Fig. 2, Table 1). The total flavonoid content in heated RRL increased significantly with increasing temperature (110 to 150°C) and time (1 to 5 hr; $p<0.001$). The total flavonoid contents in RRL and RRP were 0.83 and 1.07 mg/g, respectively. Heating at 150°C for 3 hr increased the total flavonoid content of heated RRL to 3.56 mg/g. The total flavonoid contents of heated RRL increased significantly with increasing temperature and time. The total flavonoid contents were highly correlated for all heat treatments pooled ($r=0.9229$, $p<0.001$; Table 2). Our results are consistent with those of previous studies. Stewart *et al.* (24) reported that heat treatment increases the level of free flavonols in tomatoes and tomato-based products. Also, Woo *et al.* (13) reported that total flavonoid content in onion was increased with increased heating temperatures and exposure times.

Table 2. Correlation coefficients among total polyphenol, flavonoid content, electron donating ability (EDA), and ascorbic acid equivalent antioxidant capacity (AEAC) of heated *Rehmannia radix* Libosch extract

Factor	Polyphenol	Flavonoid	EDA	AEAC
Polyphenol	1.0000	0.9229***[1)]	0.9763***	0.9432***
Flavonoid	-	1.0000	0.9174***	0.9225***
EDA	-	-	1.0000	0.9642***
AEAC	-	-	-	1.0000

[1)] ***$p<0.001$.

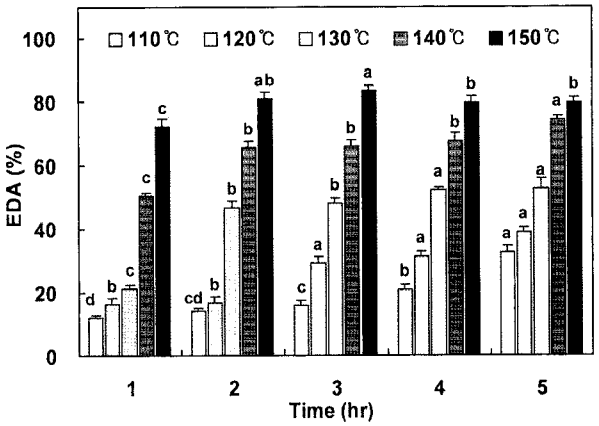

Fig. 3. Change of DPPH radical scavenging activity (electron donating ability; EDA, %) by DPPH assay at 10 mg/mL concentration of heated *Rehmannia radix* Libosch extract. *R. radix* Libosch (control), 19.44±1.90%; *R. radix* Preparata (positive control), 52.49±2.17%. Values represent the mean±SD of 3 replications. Any means in the same column followed by the different letters are significantly different ($p<0.05$) by Duncan's multiple- range test.

DPPH radical scavenging activity The radical scavenging activity toward the stable free radical DPPH was evaluated for heated RRL extracts. The antioxidant activity of heated RRL under various heating conditions was expressed as the EDA (%; Fig. 3). The EDA (%) of heated RRL was significantly affected by temperature and time, with temperature having the greater effect (Table 1). The EDA (%) of heated RRL increased significantly with increased temperature (110 to 150°C) and time (1 to 5 hr; $p<0.001$). The EDA (%) of RRL and RRP were 19.44 and 52.49%, respectively, at 10 mg/mL concentration. After heating, the EDA (%) increased to between 12.28 and 83.46% at 10 mg/mL concentration. The highest EDA (%) was 83.46%, which occurred with heating to 150°C for 3 hr. Several studies have reported effects of heating on the antioxidant activity of various foods. For example, the antioxidant activity of tomato (7), onion (13), *shiitake* mushroom (15), sweet corn (22), and citrus peel (23) increased depending on the heating temperature and duration of exposure. There were significant correlations between the EDA (%) of heated RRL and the total phenolic and total flavonoid contents ($r=0.9763$ and 0.9174, respectively, $p<0.001$; Table 2). Velioglu *et al.* (25) reported strong relationships between antioxidant activity and total phenolic content in several fruits, vegetables, and grain products.

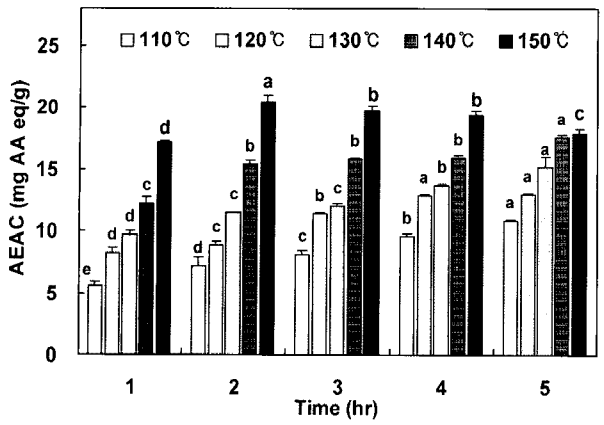

Fig. 4. Change of ascorbic acid equivalent antioxidant capacity (AEAC) by ABTS cation radical decolorization assay of heated *Rehmannia radix* Libosch extract. *R. radix* Libosch (control), 7.13±0.79 mg AA eq/g; *R. radix* Preparata (positive control), 1.74±0.73 mg AA eq/g. Values represent the mean±SD of 3 replications. Any means in the same column followed by the different letters are significantly different ($p<0.05$) by Duncan's multiple-range test.

Total antioxidant activity The ABTS cation radical scavenging activity of the heated RRL extracts under various heating conditions, expressed as the ascorbic acid equivalent antioxidant capacity (AEAC, mg AA eq/g sample) is shown in Fig. 4. The AEAC was affected by temperature and time in a manner similar to EDA (%) (Table 1). The AEAC of heated RRL increased significantly with increased temperature (110 to 150°C) and time (1 to 5 hr; $p<0.001$). The AEAC of RRL and RRP were 7.13 and 11.74 mg AA eq/g sample, respectively. After heating, the AEAC increased to between 7.18 and 20.43 mg AA eq/g sample. The highest AEAC was 20.43 mg AA eq/g sample for heated RRL at 150°C for 2 hr. Other research indicates that heating causes enhanced antioxidant activity in fruits and vegetables because of the enhancement of the antioxidant properties of naturally occurring compounds or the formation of novel compounds such as Maillard reaction products that have antioxidant activity (26,27). Therefore, it is possible that the release of phenolic compounds from RRL after heat treatment could increase its antioxidant activity. Further studies will be conducted to identify new antioxidant compounds in heated RRL.

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