Effects of roasting conditions on physicochemical properties and antioxidant activities in *Ginkgo biloba* seeds

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Abstract The roasting treatment has been used to extend the shelf life of food and improves its quality, and eliminate or reduce toxic. In this study, we investigate the changes in the physicochemical properties and antioxidant activities of *Ginkgo biloba* seeds (GBS) according to the roasting temperature and duration. As the roasting temperature and duration increased, the pH (from 7.32 to 6.31) and total cyanide content (from 1.49 to 0.70 µg/g) decreased, whereas the titratable acidity (from 0.39 to 0.84%) increased. The antioxidant activities increased rapidly at 210 °C according to the increase in the roasting temperature and duration. The 4'-O-methylpyridoxine (MPN) content in the 210 °C heat treatment group decreased by more than 70% compared to the MPN content in the control group. These results suggest that heat-treated GBS could be used in food materials and medicines for decreasing cyanide and MPN contents as well as for increasing antioxidant compound contents.

Keywords *Ginkgo biloba* seeds · Physicochemical properties · Antioxidant activities · Roasting conditions · 4'-O-methylpyridoxine

Abbreviations

GBS *Ginkgo biloba* seeds
MPN 4'-O-methylpyridoxine
DPPH 2,2-Diphenyl-1-picrylhydrazyl

ABTS 2,2'-Azino-bis-(2-ethylbenzothiazoline-6-sulfonate)
HPLC High performance liquid chromatography
AEAC L-Ascorbic acid equivalent antioxidant capacity

Introduction

Heat treatment extends the shelf life of food and improves its quality. But it has several drawbacks, such as the destruction of heat-sensitive nutrients and loss of active materials during the process. However, recent studies have reported that the contents of physiologically active substances increase as a result of various heat treatments, such as heating or blanching fruits and vegetables at approximately 100 °C, dry heat treatment including roasting at 150–200 °C, and wet heat treatment including autoclaving at 120 °C [1]. Therefore, various studies have been conducted on heat treatment processes. Among them, roasting has been reported to obtain a unique flavor and color by changing the ingredient and composition of products. It has been used to eliminate or reduce toxic and adverse effects to the human body, increase therapeutic effects, and increase the shelf life of materials [2].

Ginkgo biloba L. is a perennial plant endemic to Southeast Asia, including Korea, Japan, and China. Its seeds are drupes, with a smooth and odoriferous yellow outer seed coat and a hard and white inner seed coat having two cotyledons. The studies on *G. biloba* L. revealed flavonoids, diterpenes, sesquiterpenes, polyphenols, organic acids, and polysaccharides as its leaf components [3]. Studies on the flavonoid components of *G. biloba* leaves have been conducted to develop a drug that improves blood circulation to the brain and peripheral blood vessels by peripheral arterial dilation and platelet aggregation

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inhibition [4]. There have been several studies on *G. biloba* seeds (GBS). For example, in GBS, Wada et al. [5] identified 4'-O-methylpyridoxine (MPN), a food poisoning agent that causes vomiting, epilepsy, and convulsions by inhibiting pyridoxal kinase (a vitamin B₆-activating enzyme). Leistner and Drewke [6] also described a dose of 11–50 mg/kg of the isolated MPN triggered symptoms of poisoning in the animals. The following aspects of GBS as a food material have been studied to date: (1) antioxidant activities of seeds of GBS [7]; (2) antimicrobial activity of extracts and fractions of *G. biloba* leaves, seeds, and the outer seed coat [8]; (3) use of desserts, grazed fruit, beverages or alcoholic drinks with added GBS [9]; and (4) Functional properties of protein isolates extracted from GBS [10]. As is evident, there has been little research regarding the transformation of GBS into high value-added food materials.

Therefore, in this study, we explored the possibility of developing *G. biloba* seeds as a functional and medicinal food by evaluating the changes in physicochemical quality, antioxidant activity, and MPN composition according to roasting temperature before and after heat treatment.

Materials and methods

Materials

Fresh GBS were obtained Yeongkwang in Jeollanam-do, Korea and prepared from washed seeds by removing the hulls. DPPH (2,2-diphenyl-1-picrylhydrazyl), ABTS (2,2'-azino-bis-(2-ethylbenzothiazoline-6-sulfonate)), Folin–Ciocalteu's phenol reagent, gallic acid, L-ascorbic acid, potassium cyanide, barbituric acid, isonicotinic acid, chloramine-T, and MPN were purchased from Sigma-Aldrich (St Louis, MO, USA). All organic solvents and other chemicals were of analytical grade or high performance liquid chromatography (HPLC) grade.

Sample preparation

The roasting condition was based on a method optimized by Jinap et al. [11]. Each 150 g sample was roasted at different temperatures (150, 180, and 210 °C) for different durations (0, 15, 30, 45, and 60 min), using a temperature-controlled roaster (MK-300, JC Company, Seoul, Korea). After cooling, the roasted seeds were freeze-dried using a freeze-dryer (TFD-8503, Ilshin Lab Co., Gyeonggi, Korea) and were vacuum-packed in polyethylene film. Each sample was ground in a coffee grinder (80350, Hamilton Beach Brands, Inc., VA, USA) and then sieved through mesh No. 40 to remove larger particles, and kept at

– 30 °C before analysis. All procedures were conducted in triplicates.

Sample extraction

The lyophilized sample (10 g) was extracted with distilled water and 70% ethanol (1:10, w/v) using an ultrasonic bath (JAC-3010, Kodo Co. Ltd., Gyeonggi, Korea) for 60 min. These solutions were then centrifuged at 8000 rpm for 10 min at 4 °C. The supernatant was filtered and concentrated using a rotary evaporator (CCA-111, Eyela, Tokyo, Japan), and then freeze-dried for performing various physicochemical analyses and measuring antioxidant activity.

Physicochemical analyses

The water content of GBS according to the roasting temperature and duration was determined by drying at 105 °C until insignificant consecutive weight changes were measured [12]. The pH values were measured using a pH meter (DOCU-pH meter; Sartorius, Bohemia, NY, USA) by putting 1.0 g of distilled water extract, which was dried using a freeze-drying method, in a stirrer (KMC-130SH, Vision Scientific Co., Ltd., Daejeon, Korea), and adding 10 mL of distilled water. At the same concentration, the titratable acidity was measured by a pH meter electrode method using AOAC [13]. The samples were neutralized with 0.1 N NaOH to pH 8.2. The used amount of 0.1 N NaOH was converted into citric acid content (% w/w). The color was measured using a colorimeter (CR-300, Minolta, Osaka, Japan) for the Hunter *L** (Lightness), *a** (redness), *b** (yellowness), and ΔE^* (color difference) values. All samples were measured using a whiteboard for reference color (*L** = 93.50, *a** = 0.31, *b** = 0.32). The measurements of water content, pH, titratable acidity, and chromaticity were repeated three times and the mean values were recorded.

Antioxidant compound analyses

The detection of amounts of total cyanide and colorimetric method based on the König reaction was proposed by Epstein [14]. Each lyophilized sample (1.0 g) was homogenized with 40 mL of 0.1 M phosphoric acid and the solution was centrifuged for 20 min at 8000 rpm. A volume of 1 mL of the supernatant was transferred to a tight-capped vial and an equal amount of 4 M sulfuric acid was added. Hydrolysis was started by heating to 100 °C and continued for 50 min. The hydrolyzed sample was cooled in ice-cold water, with the stopper loosely in place, 3 mL of 3.0 M sodium hydroxide was added and after 5 min, 1 mL was added to 7 mL of 0.2 M acetate buffer at

pH 5.0. Chloramine-T (0.4 mL) was added and approximately 5 min later, 1.6 mL of isonicotinic acid/barbituric acid was added. After 60 min, the absorbance was measured at 600 nm. A calibration curve was obtained using a standard solution of potassium cyanide. The amount of total cyanide present was obtained by linear extrapolation of the data to zero time.

The total phenolic content of the GBS was measured according to the method of Singleton and Rossi [15]. Folin–Ciocalteu reagent produces a spectrophotometrically determinable molybdenum V complex as a result of reduction of the reagent by polyphenol compounds. A total of 2 mL of a 2% Na₂CO₃ solution was added to 100 µL of ethanolic extract (1.0 mg/mL) from the GBS and allowed to stand at room temperature for 3 min, followed by addition of 0.1 mL of 50% Folin–Ciocalteu reagent. The resulting mixture was incubated for 30 min at room temperature, and the absorbance was measured at 750 nm. After calibration using a standard gallic acid curve as a reference, the total phenolic content was reported in µg of gallic acid equivalent per 1.0 mg (µg GAE/mg) of sample. Triplicate measurements were taken for all samples.

The reducing power was determined according to the method of Ouchemoukh et al. [16]. A volume of 2.5 mL of ethanolic extract (1.0 mg/mL) from GBS was mixed with 2.5 mL of 200 mM sodium phosphate buffer (pH 6.6, Wako Pure Chemical Co., Osaka, Japan) and 2.5 mL of 1% potassium ferricyanide (Sigma), and the mixture was incubated at 50 °C for 20 min. After 2.5 mL of 10% trichloroacetic acid (w/v, Wako) was added, the mixture was centrifuged at 1000 rpm for 10 min. The upper layer (5 mL) was mixed with 5 mL of deionized water and 1 mL of 0.1% ferric chloride (Wako), and the absorbance was measured at 700 nm in a spectrophotometer (DU-800, Beckman Coulter Inc., CA, USA). A higher absorbance indicates a higher reducing power.

Antioxidant capacities

A DPPH solution for determination of the radical scavenging activity of *G. biloba* seeds was prepared using a modified method of Al et al. [17]. DPPH was diluted using 99.9% ethanol at 0.005 g/100 mL, followed by incubation in the dark for 2 h, which resulted in absorbance values of 1.5–1.7 at 520 nm. Additionally, 0.2 mL of an ethanolic extract (1.0 mg/mL) from GBS was added to 0.8 mL of the DPPH solution, agitated, and incubated at room temperature for 30 min, and the absorbance was measured at 520 nm. L-Ascorbic acid was added as a reference material. The DPPH radical scavenging activity was calculated as the percentage decrease in the absorbance at 520 nm relative to a blank. The positive control was 1.0% L-ascorbic acid. The percentage of the scavenging activity of the

DPPH radical was calculated using the following equation: DPPH radical scavenging activity (%) = $(A_{\text{blank}} - A_{\text{sample}}/A_{\text{blank}}) \times 100$. In this equation, A blank and A sample are the absorbances of the control and extract, respectively. All samples were analyzed in triplicate.

The total antioxidant capacity of GBS with roasting was measured using the method of Re et al. [18] with ABTS cation decolorization assays. First, 7.4 mM ABTS and 2.6 mM potassium persulfate were mixed at a 1:1 ratio, agitated, and allowed to react in the dark for 12–16 h. This mixture was then diluted with distilled water using the molar absorption coefficient so that the absorbance value was 1.4–1.5 at 735 nm. Next, 50 µL of an ethanolic extract (1.0 mg/mL) from GBS was added to 1 mL of a diluted ABTS solution and allowed to react for 60 min, after which the absorbance was measured at 735 nm. The same amount of L-ascorbic acid was added for use as a reference material. The total antioxidant capacity was expressed as antioxidant capacity relevant to L-ascorbic acid (AEAC, mg AA eq/100 g). All samples were measured in triplicate.

4'-O-methylpyridoxine (MPN) content

The MPN content of GBS with roasting was determined by HPLC using a modified method of Suh et al. [19]. An Agilent HPLC system (1200 series, Agilent Technologies, Santa Clara, CA, USA) using a Capcell column (4.6 × 250 mm, 0.5 µm, Shisedo, Tokyo, Japan) equipped with autosampler, column heater and multiple-wavelength detector (G1365B, Shimadzu, Kyoto, Japan). The binary gradient consisted of 20 mM KH₂PO₄ in distilled water (A) and 20 mM KH₂PO₄ in acetonitrile (B). The gradient was applied as follows: 0–5 min, 0% B; 5–30 min, 50% B; 30–60 min, 100% B. The injection volume of the MPN extract was 20 µL. The column temperature was set to 30 °C and the flow rate was 1 mL/min.

Statistical analyses The data are expressed as the mean ± standard deviation (SD) and were subjected to analysis of variance (ANOVA) using the statistical package for the social sciences (SPSS, version 12.0, 2004; SPSS Inc., Chicago, IL, USA). Significant differences between sample means were determined at $p < 0.05$ using Duncan's multiple comparison tests. Associations among antioxidative compound levels and activities in GBS with roasting were determined by using Pearson's correlation analysis.

Results and discussion

Physicochemical analysis Table 1 and Fig. 1 show the water content, pH, and titratable acidity of GBS according to the roasting treatment temperature (150, 180, and 210 °C) and duration (0, 15, 30, 45, and 60 min) and their

Table 1 Changes of water content, pH and titratable acidity in *Ginkgo biloba* seeds according to the roasting conditions

	Roasting temp. (°C)	Roasting time (min)					F value
		Control	15	30	45	60	
Water content (%)	150	52.12 ± 1.36 ^a	46.66 ± 2.78 ^{bA}	37.55 ± 2.77 ^{cA}	29.37 ± 2.59 ^{dA}	12.89 ± 1.55 ^{eA}	135.65***
	180	52.12 ± 1.36 ^a	43.51 ± 1.88 ^{bAB}	30.18 ± 1.51 ^{cB}	25.26 ± 2.70 ^{dA}	10.09 ± 1.79 ^{eA}	220.24***
	210	52.12 ± 1.36 ^a	39.95 ± 1.75 ^{bB}	21.99 ± 1.54 ^{cC}	7.63 ± 1.98 ^{dB}	2.62 ± 1.17 ^{eB}	691.23***
	F value	–	7.09*	44.32***	80.18***	36.51***	–
pH	150	5.63 ± 0.14 ^b	7.32 ± 0.14 ^a	7.27 ± 0.09 ^a	7.24 ± 0.10 ^{aA}	7.24 ± 0.12 ^{aA}	112.71***
	180	5.63 ± 0.14 ^c	7.18 ± 0.11 ^a	7.13 ± 0.09 ^a	6.99 ± 0.07 ^{abB}	6.80 ± 0.13 ^{bB}	102.54***
	210	5.63 ± 0.14 ^d	7.15 ± 0.08 ^a	7.06 ± 0.12 ^a	6.53 ± 0.09 ^{bC}	6.31 ± 0.12 ^{bcC}	92.81***
	F value	–	1.92 ^{NS}	3.37 ^{NS}	50.65***	43.52***	–
Titratable acidity (%)	150	1.70 ± 0.15 ^a	0.39 ± 0.08 ^{cB}	0.43 ± 0.08 ^{cB}	0.71 ± 0.11 ^{bAB}	0.75 ± 0.06 ^b	83.52***
	180	1.70 ± 0.15 ^a	0.83 ± 0.08 ^{bA}	0.81 ± 0.07 ^{bA}	0.83 ± 0.07 ^{bA}	0.84 ± 0.08 ^b	51.97***
	210	1.70 ± 0.15 ^a	0.40 ± 0.08 ^{cB}	0.37 ± 0.08 ^{cB}	0.58 ± 0.08 ^{bb}	0.70 ± 0.07 ^b	95.45***
	F value	–	31.67***	33.13***	6.51*	3.41 ^{NS}	–

Control: Non-heated *Ginkgo biloba* seed

NS not significant

Values are mean ± SD

Different letters in the same row (a–e) and column (A–C) are significantly different at $p < 0.05$ Significant at * $p < 0.05$ and *** $p < 0.001$, respectively **Fig. 1** Image of *Ginkgo biloba* seeds according to the roasting conditions. *Ginkgo biloba* seeds roasted at (A) 150 °C, (B) 180 °C, and (C) 210 °C

appearance. The GBS (Fig. 1) showed a darker shade of umber in the 210 °C heat treatment group than in the 150 and 180 °C heat treatment groups. In the 180 and 210 °C heat treatment groups, the groups that received heat treatment for 45 and 60 min showed a vivid brown color, thus indicating that the seeds of these groups were inedible. This change in appearance may have resulted from the rapid increase in brown pigment production at these roasting temperatures, which greatly support the caramelization reaction of monosaccharides and the

Maillard reaction between amino components and reducing sugars.

In the case of heat treatment for 60 min, the water contents depending on roasting heat treatment temperature were 12.89 ± 1.55 , 10.09 ± 1.79 and $2.62 \pm 1.17\%$ in the 150, 180, and 210 °C heat treatment groups, respectively. Thus, compared to the control group that saw a decrease in water content of $52.12 \pm 1.36\%$, a higher decrease of 75% was observed in this treatment. These results indicate that the water content decrease was more significantly affected by the temperature than by the duration. These results were similar to those obtained by Emily et al. [20] in the study using raw soybeans. Yoon and Kim [21] reported that as the heat treatment temperature increased, the elution of water-soluble components increased owing to the volume expansion resulting from the thermal contact-induced water evaporation increase. Thus, the higher the heat treatment temperature is, the stronger is the relationship between physicochemical and antioxidative component changes.

The pH value increased from 5.63 ± 0.14 before the heat treatment (control group) to 7.32 ± 0.14 after the heat treatment. However, in the case of the 60 min heat treatment, the pH values in the 180 and 210 °C heat treatment groups were 6.80 ± 0.13 and 6.31 ± 0.12 , respectively, thus showing a decreasing trend ($p < 0.05$).

The titratable acidity decreased from $1.70 \pm 0.15\%$ before the heat treatment (control group) to

0.39 ± 0.08 – $0.83 \pm 0.08\%$ after the heat treatment; however, it was significantly increased (0.70 ± 0.07 – $0.84 \pm 0.08\%$) from the 45 min heat treatment ($p < 0.05$), thus showing a contrasting trend with pH change.

Bae et al. [22] reported that the pH decreased in the hot water extract of *Liriopsis* tubers and Korean red ginseng as the heat treatment temperature increased. Żyżelewicz et al. [23] also showed that the pH of roasted cocoa beans decreased; however, its acidity increased with increased heat treatment temperature. Their results demonstrated the same trend as the results of this study. As the heat treatment temperature and duration increased, the aldehyde functional group of reducing sugar (an organic acid precursor) was converted to the carbonyl functional group owing to the oxidation by heat. It is determined that as the basic amino acid bound to the sugar and was involved in the Maillard reaction, the soluble basic amino acid was gradually decreased, thus leading to the decreased pH and increased acidity [24].

Table 2 shows the chromaticity and color difference (ΔE^*) of GBS depending on heat treatment temperature and duration. The L^* value (Lightness) was the highest (95.59 ± 2.55) in the control group before heat treatment, but it significantly decreased (77.76 ± 1.97 – 86.30 ± 1.47)

as the heat treatment temperature and duration increased ($p < 0.05$). In addition, as the heat treatment temperature and duration increased, the a^* value (redness) was significantly increased and the b^* value (yellowness) was significantly decreased ($p < 0.05$). Based on the degrees of color change (trace, 0.0–0.5; slight, 0.5–1.5; noticeable, 1.5–3.0; appreciable, 3.0–6.0; much, 6.0–12.0; very much, > 12.0) presented by the National Bureau of Standards' regulations [25], all GBS showed color difference values (ΔE^*) of 10 or more, thereby belonging to the “very much” category, after heat treatment, regardless of the heat treatment temperature and duration. These results were similar to those of Shakerardekani et al. [26] in terms of the soluble browning materials of pistachio kernels according to heat treatment temperature and duration. These previous studies showed that the L^* and b^* values decreased and the a^* value increased with heat treatment. Therefore, as the heat treatment temperature and duration increased because of roasting, the chromaticity of GBS was more affected by redness than yellowness.

Additionally, these results show that Maillard reaction and roasting progress was accelerated, thus increasing the brown pigment; the temperature and duration increased as well. Hodge [27] reported that dark brown polymers were produced by the condensation and polymerization of low-

Table 2 Changes of Hunter's color L^* , a^* , b^* and ΔE^* value in *Ginkgo biloba* seeds according to the roasting conditions

Roasting temp. (°C)	Hunter's color value	Roasting time (min)					F value
		Control	15	30	45	60	
L^*	150	95.59 ± 2.55^a	86.55 ± 1.25^b	88.46 ± 2.99^b	87.27 ± 1.04^{bA}	86.30 ± 1.47^{bA}	11.07**
	180	95.59 ± 2.55^a	84.69 ± 1.72^{bc}	85.51 ± 2.98^b	81.69 ± 1.69^{bcB}	81.32 ± 0.97^{cB}	22.68***
	210	95.59 ± 2.55^a	84.66 ± 2.03^b	83.69 ± 2.79^b	79.18 ± 1.17^{cB}	77.76 ± 1.97^{cC}	31.21***
	F value	–	1.23 ^{NS}	2.03 ^{NS}	29.11***	23.72***	–
a^*	150	-0.23 ± 0.09^c	3.25 ± 0.32^{aA}	2.74 ± 0.24^{bB}	3.07 ± 0.15^{abB}	2.84 ± 0.15^{bC}	145.72***
	180	-0.23 ± 0.09^d	2.59 ± 0.22^{cB}	2.32 ± 0.25^{cB}	3.62 ± 0.49^{bB}	5.64 ± 0.15^{aB}	182.48***
	210	-0.23 ± 0.09^e	2.68 ± 0.25^{dB}	4.22 ± 0.93^{cA}	6.13 ± 0.27^{bA}	7.19 ± 0.35^{aA}	114.01***
	F value	–	5.43*	9.02*	72.77***	262.81***	–
b^*	150	2.91 ± 1.07^a	-3.37 ± 1.29^b	-2.16 ± 1.47^b	-2.33 ± 1.40^b	-1.28 ± 0.55^{bA}	12.38***
	180	2.91 ± 1.07^a	-1.58 ± 1.38^b	-1.16 ± 0.57^b	-1.01 ± 1.00^b	-2.06 ± 1.01^{bA}	11.09**
	210	2.91 ± 1.07^a	-3.00 ± 1.01^b	-2.40 ± 0.32^b	-3.52 ± 0.77^b	-5.17 ± 0.41^{cB}	16.15***
	F value	–	1.76 ^{NS}	1.49 ^{NS}	4.00 ^{NS}	25.65***	–
ΔE^*	150	–	11.5	9.2	10.4	10.6	–
	180	–	12.1	10.9	15.0	16.2	–
	210	–	12.8	13.8	18.7	20.9	–

Control: Non-heated *Ginkgo biloba* seed

NS not significant

Values are mean $\pm$ SD

Different letters in the same row (a–e) and column (A–C) are significantly different at $p < 0.05$

Significant at * $p < 0.05$, ** $p < 0.01$, and *** $p < 0.001$, respectively

molecular-weight melanoidin over a long time at high temperatures.

Antioxidant compound analyses

Table 3 shows the total cyanide, total phenolic contents, and reducing power of GBS depending on the heat treatment temperature and duration. The total cyanide content significantly decreased with increasing temperature and duration ($p < 0.05$). Compared with the control, the 210 °C heat treatment group after a duration 30 min showed a significant reduction of over 50% (1.74 ± 0.04 – 0.78 ± 0.14 µg/g). In sorghum cultivars seeds, Ahmed et al. [28] reported that heat treatment of germinated seeds significantly reduced cyanide content and only traces were detected in the final product. Cardoso et al. [29] reported that heating cassava samples reduced the cyanide content by approximately 70%. Thus, the decreasing tendency of the cyanide content by heat treatment in these studies corroborates our results. However, Cho et al. [30] detected a small amount of cyanide content in GBS (0.25 ± 0.5 µg/g) using ion chromatography, thus showing a different result from ours in terms of the cyanide content. This may have resulted from the different

measurement accuracies of the colorimetric method using acid hydrolysis and ion chromatography to measure cyanide.

The total phenolic content significantly increased in the 210 °C heat treatment group with increasing the heat treatment duration ($p < 0.05$). However, no significant difference was observed in the 150 and 180 °C heat treatment groups ($p < 0.05$). The total phenolic content with 60 min duration was significantly increased in the 210 °C heat treatment group (9.83 ± 0.32 µg GAE/mg) compared to the control group (5.71 ± 0.34 µg GAE/mg). Also, at high heat treatment temperature, the total phenolic content significantly increased after 45 min duration ($p < 0.05$). Mikašauskaitė et al. [31] reported the total phenolic content of GBS as 33.8 mg RE/g, which is different from that reported in the present study. Ellnain-Wojtaszek [32] reported that GBS contain phenolic acids, such as protocatechuic acid, vanillic acid, *p*-hydroxybenzoic acid, ferulic acid, caffeic acid, protocatechuic acid, and *p*-coumaric acid. The total phenolic content increase in the above results may have resulted from the conversion of a significant amount of polymer polyphenols, which were strongly ester-bonded to polysaccharides and oligosaccharides, into free polyphenols on heating [33]. Alternatively,

Table 3 Changes of total cyanide and total phenolic contents, reducing power and 4'-*O*-methylpyridoxine (MPN) content in *Ginkgo biloba* seeds according to the roasting conditions

	Roasting temp. (°C)	Roasting time (min)					F value
		Control	15	30	45	60	
Total cyanide content (µg/g)	150	1.74 ± 0.04^a	1.49 ± 0.11^{bA}	1.33 ± 0.07^{cA}	1.29 ± 0.09^{cA}	1.22 ± 0.06^{cA}	20.68***
	180	1.74 ± 0.04^a	1.15 ± 0.04^{bB}	1.16 ± 0.07^{bA}	1.16 ± 0.07^{bA}	1.17 ± 0.04^{bA}	68.53***
	210	1.74 ± 0.04^a	1.12 ± 0.14^{bB}	0.78 ± 0.14^{cB}	0.70 ± 0.17^{cB}	0.71 ± 0.14^{cB}	33.61***
	F value	–	11.60***	24.66***	21.14***	28.71***	–
Total phenolic content (µg GAE/mg)	150	5.71 ± 0.34	5.59 ± 0.54	5.27 ± 0.34	5.36 ± 0.33^B	5.52 ± 0.31^B	0.63 ^{NS}
	180	5.71 ± 0.34	5.71 ± 0.32	5.19 ± 0.32	4.98 ± 0.30^B	5.86 ± 0.32^B	4.34 ^{NS}
	210	5.71 ± 0.34^c	5.01 ± 0.30^d	4.82 ± 0.30^d	6.48 ± 0.30^{bA}	9.83 ± 0.32^{aA}	129.05***
	F value	–	2.53 ^{NS}	1.73 ^{NS}	18.70***	171.13***	–
Reducing power (A_{700nm})	150	1.21 ± 0.13^a	0.41 ± 0.11^{cB}	0.44 ± 0.09^{cB}	0.77 ± 0.08^{bB}	0.92 ± 0.04^{bC}	38.55***
	180	1.21 ± 0.13^a	0.68 ± 0.08^{cA}	0.71 ± 0.07^{cA}	0.91 ± 0.04^{bB}	1.35 ± 0.07^{aB}	39.11***
	210	1.21 ± 0.13^b	0.45 ± 0.14^{dB}	0.89 ± 0.11^{cA}	1.56 ± 0.11^{aA}	1.68 ± 0.06^{aA}	59.87***
	F value	–	5.27*	18.84***	82.09***	142.88***	–
MPN content (µg/g)	150	198.05 ± 2.07^a	188.31 ± 2.67^b	185.96 ± 2.76^b	185.04 ± 1.97^{bA}	177.86 ± 2.14^{cA}	28.98***
	180	198.05 ± 2.07^a	191.28 ± 1.69^b	188.06 ± 1.42^b	173.00 ± 1.60^{cB}	163.46 ± 2.46^{dB}	169.13***
	210	198.05 ± 2.07^a	192.06 ± 1.75^b	183.81 ± 2.31^c	155.85 ± 3.08^{dC}	52.88 ± 2.77^{eC}	1816.86***
	F value	–	2.69 ^{NS}	2.72 ^{NS}	121.65***	2301.17***	–

Control: Non-heated *Ginkgo biloba* seed

NS not significant

Values are mean $\pm$ SD

Different letters in the same row (a–e) and column (A–C) are significantly different at $p < 0.05$

Significant at * $p < 0.05$ and *** $p < 0.001$, respectively

it may have been due to the conversion of phenolic compounds of polymers into low-molecular-weight phenolic compounds because of heating at high temperatures [1].

The reducing power is the ability to donate electrons to reactive oxygen species and free radicals. The greater the reducing power and antioxidant activity are, the higher is the absorbance value. Table 3 shows the measurement results of the reducing power of GBS extracts (concentration, 1.0 mg/mL) depending on heat treatment temperature and duration. After heat treatment, the reducing power was in the absorbance range of 0.41 ± 0.11 A₇₀₀– 1.68 ± 0.06 A₇₀₀, thus being significantly decreased compared to that of the control group (1.21 ± 0.13 A₇₀₀; $p < 0.05$). Although the reducing power of the 180 and 210 °C heat treatment groups (1.35 ± 0.07 A₇₀₀ and 1.68 ± 0.06 A₇₀₀, respectively) during the 60 min heat treatment was significantly differ from that of the control group, it seemed that the reducing power was restored. The reducing power is reportedly related to the antioxidant activity and is associated with the presence of a reductone [34]. According to Terpin et al. [35], the reducing power of camelina seed was 0.578 A₇₄₀ before heat treatment and increased to 0.647 A₇₄₀ after heat treatment, thus showing a similar trend to that of the present study.

The increased reducing power may have resulted from increased phenols and antioxidative components due to heat treatment. A comparison of these results from previous studies with the results of the total phenolic content and reducing power in this study indicate that the higher the treatment temperature is, the higher is the correlation with DPPH and ABTS radical activities.

Antioxidant capacities

The electron donating ability and ascorbic acid equivalent antioxidant activity (AEAC), which were measured in GBS using DPPH and ABTS radicals at the concentration of 1.0 mg/mL, increased with increasing temperature and duration of the heat treatment, as shown in Fig. 2 ($p < 0.05$). The DPPH radical scavenging activities were 34.03 ± 2.22 and $53.07 \pm 2.37\%$, respectively, in the 180 and 210 °C heat treatment groups with 60 min duration, thus showing a significant increase compared to the activity in the control group ($24.91 \pm 2.34\%$; $p < 0.05$). The AEAC values significantly increased with increasing temperature and duration only in the 210 °C heat treatment group (38.48 ± 1.44 mg AA eq/100 g) relative to the control group values (30.40 ± 0.66 mg AA eq/100 g; $p < 0.05$). The antioxidant activities were high in the heat treatment groups with high phenol content, as shown by the total phenolic content (Table 3). These results suggest that phenolic compounds represent the antioxidative activity and that the antioxidative effect of phenolic compounds is

increased by heat treatment. These results were consistent with the reports that the antioxidant activity was significantly increased after heat treatment of camelina seed [35]. Thus, the antioxidative effect was increased as free polyphenol compounds increased after heat treatment. In addition, the heat treatment may have converted active ingredients, which had been strongly bonded to the tissue, to free forms, thus leading to the increased antioxidant effects.

4'-O-methylpyridoxine (MPN) content

Table 3 shows the content of MPN in GBS depending on heat treatment temperature and duration. As the heat treatment temperature and duration were increased, the MPN content significantly decreased compared to the control group before heat treatment ($p < 0.05$). The higher the temperature was, the greater was the decrease in the MPN content. Compared with the control group (198.05 ± 2.07 µg/g), in the 210 °C heat treatment group, the MPN content (52.88 ± 2.77 µg/g) decreased by over 70%. The MPN content showed a significant decrease in the 60 min heat treatment group at 150 °C, the 45 min heat treatment group at 180 °C, and the 30 min heat treatment group at 210 °C ($p < 0.05$), thus revealing that the MPN content decreased rapidly with increases in the heat treatment temperature. The MPN content of the control group before heat treatment reported in this study was similar to that reported by Yosimura et al. [36], whose study determined the MPN content of raw GBS to be 936 nmol/g (approximately 171.4 µg/g) and 1177 nmol/g (approximately 215.6 µg/g) using HPLC. In addition, Kobayasi et al. [37] reported that the MPN content of raw GBS significantly decreased in a 5 min boiled heat treatment group at 100 °C and the 45 s microwave heat treatment group, respectively. Thus, the decreases in the MPN content by heat treatment reported by them were similar to that reported in this study.

This may have resulted from the similarities between MPN and soluble vitamin B₆ in terms of the molecular structure and metabolic pathways [6]. Moreover, MPN is synthesized by the methylation of pyridoxine (one of soluble vitamin B₆) and pyridoxal-5'-phosphate derivatives [38]. Therefore, the MPN metabolic pathway may be similar to the metabolic pathway of vitamin B₆ reduction by heat.

Correlation between total cyanide content, total phenolic content, and antioxidant activities

To compare the contribution of individual compounds to antioxidant activities, the Pearson's correlation coefficient is showed in Table 4. The total phenolic content showed a

Fig. 2 (A) DPPH and (B) ABTS radical scavenging activity of in *Ginkgo biloba* seeds according to the roasting conditions. Each bar represents the mean $\pm$ SD of triplicated measurement. Means with

different letters in the roasting time (a–c) and roasting temperature (A–C) are significantly different at $p < 0.05$

Table 4 Pearson's correlation coefficients between total cyanide content, total phenolic content and antioxidant activities of *Ginkgo biloba* seeds according to the roasting treatment temperature (150, 180 and 210 °C)

Roasting temp. (°C)	Total cyanide content	Total phenolic content	Reducing power	DPPH	ABTS
150					
Total cyanide content	1.0000				
Total phenolic content	0.7334	1.0000			
Reducing power	0.4103	0.5876	1.0000		
DPPH	0.3432	0.7034	0.8553	1.0000	
ABTS	– 0.4517	– 0.2913	0.5070	0.4574	1.0000
180					
Total cyanide content	1.0000				
Total phenolic content	0.3231	1.0000			
Reducing power	0.4711	0.5209	1.0000		
DPPH	– 0.2435	0.4848	0.7189	1.0000	
ABTS	– 0.2461	– 0.0113	0.6674	0.8146	1.0000
210					
Total cyanide content	1.0000				
Total phenolic content	– 0.3736	1.0000			
Reducing power	– 0.2855	0.7793	1.0000		
DPPH	– 0.5997	0.9494*	0.8341	1.0000	
ABTS	– 0.6142	0.9295*	0.5804	0.9206*	1.0000

Significant at * $p < 0.05$

positive correlation with the DPPH, as well as ABTS, only in GBS roasted at 210 °C. The DPPH radical scavenging activity had a positive correlation with ABTS in roasted GBS at 210 °C. These results indicate that the major

compounds contributing to each radical scavenging activity and reducing power in GBS depending on heat treatment temperature are similar. Further studies are needed to

elucidate the contribution of each compound to the antioxidant activity of GBS.

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References

- Choi Y, Lee SM, Chun J, Lee HB, Lee J. Influence of heat treatment on the antioxidant activities and polyphenolic compounds of shiitake (*Lentinus edodes*) mushroom. *Food Chem.* 99: 381–387 (2006)
- Wandsnider L. The roasted and the boiled: food composition and heat treatment with special emphasis on pitheath cooking. *J. Anthropol. Archaeol.* 16: 1–48 (1997)
- Chan PC, Xia Q, Fu PP. *Ginkgo Biloba* leave extract: biological, medicinal, and toxicological effects. *J. Environ. Sci. Health C Environ. Carcinog. Ecotoxicol Rev.* 25: 211–244 (2007)
- Kleijnen J, Knipschild P. *Ginkgo biloba*. *Lancet* 340: 1136–1139 (1992)
- Wada K, Ishigaki S, Ueda K, Sakata M, Haga M. An antivitamin B6, 4'-methoxypyridoxine from the seed of *Ginkgo biloba* L. *Chem. Pharm. Bull.* 33: 3555–3557 (1985)
- Leistner E, Drewke C. *Ginkgo biloba* and Ginkgotoxin. *J. Nat. Prod.* 73: 86–92 (2010)
- Tommasi F, Paciolla C, de pinto MC, De Gara L. Effects of storage temperature on viability, germination and antioxidant metabolism in *Ginkgo biloba* L. seeds. *Plant Physiol. Biochem.* 44: 359–368 (2006)
- Park SB, Cho GS. Antimicrobial activity of extracts and fractions of *Ginkgo biloba* leaves, seed and outer seedcoat. *J Korean Soc Food Sci Nutr* 40: 7–13 (2011)
- Tredici PD. Ginkgos and people - a thousand years of interaction. *Arnoldia* 51: 2–15 (1991)
- Deng Q, Wang L, Wei F, B Xie, F Huang, W Huang, J Shi, Q Huang, B Tian, S Xue. Functional properties of protein isolates, globulin and albumin extracted from *Ginkgo biloba* seeds. *Food Chem.* 124: 1458–1465 (2011)
- Jinap S, Wan Rosli WR, Russly AR, Nordin LM. Effect of roasting time and temperature on volatile component profiles during nib roasting of cocoa beans (*Theobroma cacao*). *J. Sci. Food Agric.* 77: 441–448 (1998)
- AOAC. Official Methods of Analysis. 17th ed. Method 934.01. Association of Official Analytical Communities, Gaithersburg, MD, USA (2006)
- AOAC. Official Methods of Analysis. 14th ed. Method 942.15. Association of Official Analytical Communities, Arlington, VA, USA (1984)
- Epstein J. Estimation of microquantities of cyanide. *Anal. Chem.* 19: 272–274 (1947)
- Singleton VL, Rossi JA Jr. Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagent. *Am. J. Enol. Viticult.* 16: 144–158 (1965)
- Ouchemoukh S, Hachoud S, Boudraham H, Mokrani A, Louaiche H. Antioxidant activities of some dried fruits consumed in Algeria. *LWT-Food Sci. Technol.* 49: 329–332 (2012)
- Al ML, Dezmirean D, Adela M, Otilia B, Laslo L, Bogdanov S. Physicochemical and bioactive properties of different floral origin honeys from Romania. *Food Chem.* 112: 863–867 (2009)
- Re R, Pellegrini N, Proteggente A, Pannala A, Yang M, Rice-Evans C. Antioxidant activity applying an improved ABTS radical cation decolorization assay. *Free Radic. Biol. Med.* 26: 1231–1237 (1999)
- Suh JH, Yang DH, Lee BK, Eom HY, Kim UY, Kim JH, Lee HY, Han SB. Simultaneous determination of B group vitamins in supplemented food products by high performance liquid chromatography-diode array detection. *Bull Korean Chem. Soc.* 32: 2648–2656 (2011)
- Emily LB, Terri DB, Lester AW. Effect of cultivar and roasting method on composition of roasted soybeans. *J. Sci. Food Agric.* 89: 821–826 (2009)
- Yoon SK, Kim WJ. Effects of roasting conditions on quality and yields of barley tea. *Korean J Food Sci Technol* 21: 575–582 (1987).
- Bae KM, Park SH, Jung KH, Kim MJ, Hong SH, Song YO, Lee HS. Effects of roasting conditions on physicochemical properties and sensory properties of *Liriopsis* Tuber. *J Korean Soc Food Sci Nutr* 39: 1503–1508 (2010)
- Żyżelewicz D, Budryn G, Krysiak W, Oracz J, Nebesny E, Bojczuk M. Influence of roasting conditions on fatty acid composition and oxidative changes of cocoa butter extracted from cocoa bean of Forastero variety cultivated in Togo. *Food Res. Int.* 63: 328–343 (2014)
- Saunders J, Jervis F. The role of buffer salts in non-enzymatic browning. *J. Sci. Food Agric.* 17: 245–249 (1966)
- Wood LA, Shouse PJ. Standard reference materials: Use of standard light-sensitive paper for calibrating carbon arcs used in testing textiles for colorfastness to light (Special Publication 260-41). National Bureau of Standards, Washington, DC, USA, pp. 1–24 (1972)
- Shakerdekani A, Karim R, Ghazali HM, Chin NL. Effect of roasting conditions on hardness, moisture content and colour of pistachio kernels. *International Food Research journal* 18: 723–729 (2011)
- Hodge JE. Dehydrated foods: chemistry of browning reactions in model systems. *J. Agric. Food Chem.* 1: 928–943 (1953)
- Ahmed SB, Mahgoub SA, babiker BE. Changes in tannin and cyanide contents and diastatic activity during germination and the effect of traditional processing on cyanide content of sorghum cultivars. *Food Chem.* 56: 159–162 (1996)
- Cardoso AP, Mirione E, Ernesto M, Massaza F, Cliff J, Haque MR, Bradbury JH. Processing of cassava roots to remove cyanogens. *J. Food Compos. Anal.* 18: 451–460 (2005)
- Cho HJ, Do BK, Shlm SM, Kwon HJ, Lee DH, Nah AH, Chol YJ, Lee SY. Determination of cyanogenic compounds in edible plants by ion chromatography. *Toxicol. Res.* 29: 143–147 (2013)
- Mikašauskaitė J, Ragažinskienė O, Maruška A. Variation of total amount of phenolic compounds, radical scavenging activity and volatile compounds of *Liriodendron tulipifera* L. and *Ginkgo biloba* L. leaves extracts during different vegetation periods. *Biologija* 59: 175–186 (2013)
- Ellnain-Wojtaszek M. Phenolic acids from *Ginkgo biloba* L. Part I. Qualitative analysis of free and liberated by hydrolysis phenolic acids. *Acta Pol. Pharm.* 54: 225–228 (1997)
- Xu B. and Chang SKC. Total phenolic, phenolic acid, anthocyanin, flavan-3-ol, and flavonol profiles and antioxidant properties of pinto and black beans (*Phaseolus vulgaris* L.) as affected by thermal processing. *J. Agric. Food Chem.* 57: 4754–4764 (2009)
- Yoshino M, Murakami K. Interaction of iron with polyphenolic compounds, application to antioxidant characterization. *Anal. Biochem.* 257: 40–44 (1998)
- Terpinc P, Polak T, Poklar N, Ulrih NP, Abramovic H. Effect of heat treatment of camelina (*Camelina sativa*) seeds on the antioxidant potential of their extracts. *Food Chem.* 97: 472–479 (2006)

36. Yoshimura T, Uda N, Morita J, Jinyu Z, Sasaki K, Kobayashi D, Wada K, Hori Y. High performance liquid chromatographic determination of ginkgotoxin and ginkgotoxin-5'-Glucoside in *Ginkgo biloba* seeds. J. Liq. Chromatogr. Related Technol. 29: 605–616 (2016)
37. Kobayashi, D, Yoshimura T, John A, Sasaki K, Wada K. Toxicity of 4'-O-methylpyridoxine-5'-glucoside in *Ginkgo biloba* seeds. Food Chem. 126: 1198–1202 (2011)
38. Fiehe K, Arenz A, Drewke C, Hemscheidt T, Williamson RT, Leistner E. Biosynthesis of 4'-O-methylpyridoxine (Ginkgotoxin) from primary precursors. J. Nat. Prod. 63: 185–189 (2000)