Nutraceuticals and antioxidant properties of *Lonicera japonica* Thunb. as affected by heating time

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**ABSTRACT**

This study investigated the effects of different heating times (30–150 min) at 100°C on nutraceuticals and antioxidant properties of *Lonicera japonica* Thunb. (LJ). Total phenolic, phenolic acid (chlorogenic acid, caffeic acid, 4,5-dicaffeoylquinic acid, and 3,5-dicaffeoylquinic acid) and flavonoids (rutin, quercetin, and luteolin) in LJ were significantly increased after heat treatments. Antioxidant activities, such as DPPH radical scavenging activity, ABTS radical scavenging activity, FRAP and reducing power, of LJ, were improved after heating. Antioxidant activities were positively correlated with total phenolic, total flavonoid, chlorogenic acid, caffeic acid, and quercetin contents.

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**Introduction**

*Lonicera japonica* Thunb. (LJ), belonging to the Caprifoliaceae family, is native in East Asia. Nowadays, LJ has been widely used in the food industry because of its health benefits, such as antioxidant activity,\[^1^–^3^\] anti-inflammatory activity,\[^2^,^4^\] and antibacterial activity.\[^5^\] It has been reported that LJ has numerous phytochemicals, and the main active constituents are phenolic compounds such as phenolic acids and flavonoids.\[^6^\] Among them, chlorogenic acid, a phenolic acid is, a standard compound used to assess the chemical quality of LJ.\[^7^\]

Most phenolic compounds form covalent bonds with cellular structures, including cellulose, hemicellulose, and lignin, and are present in bound forms in plants. Especially, bound phenolic acids can form ester linkages with the structural carbohydrates and ether linkages with lignin.\[^8^\] Since bound phenolic compounds can not function as antioxidants, one must break down those linkages to convert bound phenolics into free forms. Acosta-Estrada et al.\[^9^\] demonstrated that several food-processing methods, such as heating, malting, and fermentation, can increase the liberation of bound phenolic compounds in a food matrix and can improve the biological activities of food.

Heating is a common method employed in food processing. In general, heating causes the loss of heat-sensitive compounds, thus reducing the nutritional quality.\[^10^\] Therefore, heating can be an unfavorable method in food processing, because consumers demand high-quality, cheap, and convenient products with great nutritional value.\[^11^\]

However, improvement of phenolic compounds contents and antioxidant properties of various heated foods has been reported. According to Kim et al.\[^12^\] heating at 100°C for 30–90 min significantly improved total phenolic contents in grape-seed extracts. Furthermore, significant increases in the FRAP value of grape-seed flour were observed after heating at 100°C for 50–60 min.\[^13^\] Heating can improve nutrition values in various foods by destroying cell structures and can lead to the release of bound phenolics, which implies great bioaccessibility.\[^14^\]
To date, there is limited information on how nutraceuticals and antioxidant properties of LJ are affected by heating for different heating times. Therefore, the objectives of this study were to elucidate the effect of heating time (30–150 min) on (1) individual phenolic acid contents (chlorogenic acid, caffeic acid, 4,5-dicaffeoylquinic acid, and 3,5-dicaffeoylquinic acid) and individual flavonoids (rutin, quercetin, and luteolin) in LJ and (2) the antioxidant properties (DPPH radical scavenging activity, ABTS radical scavenging activity, FRAP value, and reducing power) of LJ.

Materials and methods

Materials

The whole plants of LJ were purchased from an online market (Goseong, Korea) and were deposited in the herbarium of the Department of Food and Nutrition, Kyunghee University under the voucher number KCL213. Chlorogenic acid, caffeic acid, 4,5-dicaffeoylquinic acid, 3,5-dicaffeoylquinic acid, rutin, quercetin, luteolin, quinic acid, malic acid, shikimic acid, citric acid, and succinic acid were purchased from Sigma-Aldrich Chemical Co. (St. Louis, MO, USA). All other chemicals and reagents were HPLC or analytical grade.

Heat treatment

LJ samples (12 g, dw), in Erlenmeyer flasks, were sealed and equilibrated at 4°C for 24 h. Samples were then heated at 100°C for different periods (30, 60, 90, 120, and 150 min) in a dry oven (Thermo Stable EOF-155, DAIHAN Scientific Co., Korea). All samples were stored in a freezer (-18°C) until analysis.

pH and soluble solids

The pH values of heat-treated LJ samples were measured by a pH-meter (ORION 3 STAR pH Benchtop, Thermo Scientific, Waltham, MA, USA). Soluble solids of heat-treated LJ were estimated with a portable refractometer (PAL-alpha, ATAGO CO. LTD., Tokyo, Japan) previously adjusted to zero with distilled water.

Chlorophyll contents

Chlorophyll in heat-treated LJ samples was measured according to Medina-Meza et al.\textsuperscript{[15]}, with slight modifications. One gram of sample was homogenized at 15,000 rpm in 25 mL of 80% (v/v) acetone solution for 1 min and centrifuged at 3,500 rpm for 10 min. The supernatant was obtained and absorbance was measured at 647 and 663 nm using a spectrophotometer (Thermo Scientific, Vantaa, Finland). Chlorophyll a, b, and total chlorophyll contents were calculated as follows:

\[
\text{Chlorophyll a (C}_a\text{, ug/g)} = 12.25 \times A_{663} - 2.35 \times A_{647} \tag{1}
\]

\[
\text{Chlorophyll b (C}_b\text{, ug/g)} = 21.50 \times A_{647} - 5.10 \times A_{663} \tag{2}
\]

Where \(A_{663}\) is the absorbance at 663 nm and \(A_{647}\) is the absorbance at 647 nm.

Color values

The color values of heat-treated LJ samples were measured using a Minolta Chroma Meter CR-400 (Konica Minolta, Tokyo, Japan) calibrated using a white tile. The results were expressed as CIE \(L^*\) (lightness), \(a^*\) (redness), and \(b^*\) (yellowness). The net color difference (\(\Delta E\)), hue angle (\(H^\circ\)), and chroma or saturation index (\(C^*\)) were calculated using \(L^*, a^*,\) and \(b^*\) values as follows:
ΔE = \sqrt{(ΔL*)^2 + (Δa*)^2 + (Δb*)^2} \tag{3}

H = \tan^{-1}(b*/a*) \tag{4}

C* = \sqrt{(a*)^2 + (b*)^2} \tag{5}

**Total phenolic and total flavonoid contents**

The total phenolic contents in heat-treated LJ samples were measured using the Folin-Ciocalteau reagent method described by Kähkönen et al.\textsuperscript{[16]}, with slight modifications. Briefly, 10 mL of a 70% (v/v) methanol solution was added to 0.1 g of heat-treated LJ samples. Each suspension was vortexed for 1 min, sonicated for 30 min at room temperature and then centrifuged at 3,500 rpm for 20 min. Then, the supernatant was diluted 5 times with distilled water. The sample solution (0.5 mL) was mixed with 0.5 mL of 1 N Folin-Ciocalteau reagent. After 3 min, 1.5 mL of 10% (w/v) sodium carbonate solution was added to the mixture. Next, the mixture was incubated in darkness at room temperature for 60 min. The absorbance of the mixture was measured at 725 nm using a spectrophotometer (Thermo Scientific, Vantaa, Finland). Gallic acid (0–100 μg/mL) was used as a standard, and the results were expressed as micrograms of gallic acid equivalent per 100 mg of sample (μg GAE/100 mg).

The total flavonoids in the heat-treated LJ samples were measured using the aluminum-chloride colorimetric method described by Zhu et al.\textsuperscript{[17]}, with slight modifications. Briefly, 10 mL of 70% (v/v) methanol solution was added to 0.1 g of heat-treated LJ samples. Each suspension was vortexed for 1 min, sonicated for 30 min at room temperature, and then centrifuged at 3,500 rpm for 20 min. Then, the supernatant was diluted 5 times with 70% (v/v) methanol. The sample solution (0.3 mL) was mixed with an equal volume of distilled water followed by an addition of 30 μL of 5% (w/v) NaNO\textsubscript{2}. The mixture was incubated at room temperature for 5 min and the 60 μL of 10% (w/v) AlCl\textsubscript{3} was added to the mixture. Next, the mixture was incubated at room temperature for 5 min, and then 200 μL of 1 M NaOH was added to the mixture. The absorbance of the mixture was measured at 500 nm using a spectrophotometer (Thermo Scientific, Vantaa, Finland). Rutin (0–200 μg/mL) was used as a standard, and the results were expressed as micrograms of rutin equivalent per 100 mg of sample (μg RE/100 mg).

**Measurement of individual phenolic acids and flavonoids using RP-HPLC**

*Sample preparation for measuring individual phenolic acids and flavonoids:* Accurately quantified 0.1 g of heat-treated LJ samples were mixed with 5 mL of 70% (v/v) methanol solution. Each suspension was vortexed for 1 min, sonicated for 30 min at room temperature, and then centrifuged at 3,500 rpm for 20 min. The supernatant was filtered through a 0.45 μm membrane filter (Millipore, Ireland) prior to RP-HPLC analysis.

*Quantification of individual phenolic acids using RP-HPLC:* An Agilent 1,200 HPLC system (Agilent Technologies, Palo Alto, USA) equipped with a G1322A vacuum degasser, a G1311A quaternary pump, a G1315D diode array detector (DAD), a G1316A column compartment, and a G1313A autosampler was used to obtain HPLC chromatograms for heat-treated samples. The system was controlled with Agilent Chemstation software (Agilent Technologies, Palo Alto, USA). Chromatographic separations were done on a Zorbax Eclipse XDB-C18 reversed-phase column (4.6×250 mm i.d., 5 um) maintained at 25°C. The mobile phase was 0.2% (v/v) phosphoric acid aqueous water (A) and methanol (B). Gradient elution was done as follows: 0–30 min, 30–60% B. The flow rate was 1 mL/min and the injection volume was 10 μL.

Individual phenolic acids (chlorogenic acid, caffeic acid, 4,5-dicaffeoylquinic acid, and 3,5-dicaffeoylquinic acid) were verified at a wavelength of 340 nm and used for RP-HPLC
quantification. The individual phenolic acids in heat-treated LJ samples were confirmed by comparing their retention times and UV spectra with those of standards using a concentration range of 10 to 500, 0.1 to 10, 1 to 100, and 10 to 250 ug/mL, respectively. The $R^2$ values were 0.99996, 0.99991, 0.99996, and 0.99996, respectively.

Quantification of individual flavonoids using RP-HPLC: An Agilent 1,200 HPLC system (Agilent Technologies, Palo Alto, USA) equipped with a G1322A vacuum degasser, a G1311A quaternary pump, a G1315D diode array detector (DAD), a G1316A column compartment, and a G1313A autosampler was used to obtain HPLC chromatograms for heat-treated samples. The system was controlled with Agilent Chemstation software (Agilent Technologies, Palo Alto, USA). Chromatographic separations were done on a Zorbax Eclipse XDB-C18 reversed-phase column (4.6×250 mm i.d., 5 um) maintained at 25°C. The mobile phase was 0.2% (v/v) phosphoric acid aqueous water (A) and methanol (B). Gradient elution was done as follows: 0–30 min, 30–60% B. The flow rate was 1 mL/min and the injection volume was 10 uL.

Individual flavonoids (rutin, quercetin, and luteolin) were verified at a wavelength of 340 nm and used for RP-HPLC quantification. Rutin, quercetin, and luteolin in heat-treated LJ samples were confirmed by comparing their retention times and UV spectra with those of standards using a concentration range of 1 to 50, 0.1 to 5, and 0.1 to 10 ug/mL, respectively. The $R^2$ values were 0.99953, 0.99388, and 0.99990, respectively.

Measurement of individual organic acids using HPLC

Sample preparation for measuring individual organic acids: Accurately quantified 0.1 g of heat-treated LJ samples were mixed with 5 mL of distilled water. Each suspension was vortexed for 1 min, sonicated for 30 min at room temperature and then centrifuged at 3,500 rpm for 20 min. The supernatant was filtered through a 0.45 um membrane filter (Millipore, Ireland) prior to HPLC analysis.

Quantification of organic acids using HPLC: The same Agilent 1,200 HPLC system as described was used to obtain HPLC chromatograms for heat-treated samples. Chromatographic separations were done on a Zorbax SB-Aq column (4.6×150 mm i.d., 5 um) maintained at 25°C. The mobile phase was 200 mM aqueous phosphate buffer (pH 2.0)/acetonitrile (99:1, v/v). The flow rate was 0.6 mL/min and the injection volume was 10 uL.

Quinic acid, malic acid, shikimic acid, citric acid, and succinic acid were verified at a wavelength of 214 nm, with a reference wavelength of 360 nm, and used for HPLC quantification. Organic acid compounds in heat-treated LJ samples were confirmed by comparing their retention times and UV spectra with those of standards using a concentration range of 20 to 1,000, 10 to 500, 1 to 10, 10 to 500, and 10 to 200 ug/mL, respectively. The $R^2$ values were 0.99998, 1.00000, 0.99998, 0.99999 and 0.99977, respectively.

Measurement of antioxidant properties

Sample preparation for measuring antioxidant properties: Accurately quantified 0.1 g of heat-treated LJ samples were mixed with 10 mL of 70% (v/v) methanol solution. Each suspension was vortexed for 1 min, sonicated for 30 min at room temperature, and then centrifuged at 3,500 rpm for 20 min. The supernatant was diluted 10 times with 70% (v/v) methanol and was used for subsequent analysis.

DPPH radical scavenging activity: The 2,2-Diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activities of heat-treated LJ samples were measured by the method of Blois\(^{18}\), with slight modifications. The sample solution (0.2 mL) was mixed with 0.6 mL of 200 mM DPPH solution. The mixture was incubated in darkness at room temperature for 15 min. The absorbance of the mixture was measured at 517 nm using a spectrophotometer (Thermo Scientific, Vantaa, Finland). Ascorbic acid (0–60 ug/mL) was used as an antioxidant standard. The DPPH radical scavenging activity was calculated as follows:
DPPH radical scavenging activity (\(\%\)) = \([A_0 - A_1]/A_0 \times 100\) 

Where \(A_0\) is the absorbance of the blank and \(A_1\) is the absorbance of the test compound.

**ABTS radical scavenging activity:** The 2,2’-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) radical scavenging activities of heat-treated LJ samples were measured by the method of Shalaby et al.\(^{[19]}\), with slight modifications. The radical cation was prepared by mixing 7 mM ABTS stock solution with 2.45 mM potassium persulfate (1:1, v/v), leaving the mixture for 12–16 h until the reaction was completed and the absorbance was stable. The ABTS\(^+\) solution was diluted with methanol to the absorbance of 0.700 ± 0.005 at 734 nm. Then, the sample solution (0.1 mL) was mixed with 0.9 mL of ABTS\(^+\) solution. The mixture was incubated in darkness at room temperature for 6 min. The absorbance of the mixture was measured at 734 nm using a spectrophotometer (Thermo Scientific, Vantaa, Finland). Trolox (0–90 \(\mu\)g/mL) was used as an antioxidant standard. The ABTS radical scavenging activity was calculated as follows:

\[
\text{ABTS radical scavenging activity (\%)} = \left[\frac{A_0 - A_1}{A_0}\right] \times 100
\]

Where \(A_0\) is the absorbance of the blank and \(A_1\) is the absorbance of the test compound.

**Ferric reducing antioxidant power (FRAP) assay:** Ferric reducing antioxidant powers (FRAP) of heat-treated LJ samples were measured by the method of Benzie and Strain\(^{[20]}\), with slight modifications. FRAP reagent was prepared by mixing 300 mM sodium acetate buffer (pH 3.6) with 10 mM 2,4,6-tripyridyl-s-triazine (TPTZ) solution in 40 mM HCl and 20 mM FeCl\(_3\) solution at a ratio of 10:1:1 (v/v/v) and was warmed at 37°C. Then, the sample solution (0.15 mL) was mixed with 2.85 mL of FRAP reagent and incubated in darkness at 37°C for 15 min. The absorbance of the mixture was measured at 593 nm using a spectrophotometer (Thermo Scientific, Vantaa, Finland). FeSO\(_4\) · 7H\(_2\)O (0–1 mM) was used as a standard, and the results were expressed as millimole of Fe\(^{2+}\) ·7H\(_2\)O equivalent per 100 mg of sample (mM Fe\(^{2+}\)·7H\(_2\)O/100 mg).

**Reducing power:** The reducing powers of heat-treated LJ samples were measured by the method of Sanjukta et al.\(^{[21]}\), with slight modifications. Briefly, the sample solution (0.1 mL) was mixed with 0.9 mL of 200 mM phosphate buffer (pH 6.6) and 1% (w/v) potassium ferric cyanide. The mixture was incubated at 50°C for 20 min. Then, 0.9 mL of 10% (w/v) trichloroacetic acid was added to the mixture and centrifuged at 3,500 rpm for 20 min. The supernatant (0.9 mL) was mixed with 0.9 mL of distilled water and 0.9 mL of 0.1% (w/v) FeCl\(_3\). The absorbance of the mixture was measured immediately at 700 nm using a spectrophotometer (Thermo Scientific, Vantaa, Finland). Ascorbic acid (0–100 \(\mu\)g/mL) was used as a standard, and the results were expressed as micrograms of ascorbic acid equivalent per one milligram of sample (\(\mu\)g AAE/mg).

**Statistical analysis**

All statistical analyses were done using SAS version 9.4 (SAS Institute, Inc., Cary, NC, USA). Analysis of variance (ANOVA) was done using the general linear models (GLM) procedure to find significant differences between the samples. Means were compared by using Fisher’s least significant difference (LSD) procedure. Significance was defined at the 5% level. In addition, Pearson correlation coefficients between the samples were calculated. The CORR procedure was used to obtain correlation coefficients.

**Results and discussion**

**pH values and soluble solid contents**

The pH values and soluble solid contents of LJ samples heated for 30–150 min are given in Table 1. The pH values of heat-treated LJ samples were almost constant during heating for 60 min but began to decrease significantly after 90 min of heating. The decrease in pH values after heating for more than 90 min can be attributed to the increasing organic acid contents in LJ. Soluble solid contents in LJ were not significantly affected by heating for different heating times (30–150 min).
Chlorophyll contents

Chlorophylls are the important group of biologically active compounds with health promoting properties. Structurally, chlorophylls are composed of a porphyrin ring replaced by a centrally bound Mg$^{2+}$ atom and a highly hydrophobic esterified phytol tail ($C_{20}H_{39}$). This structure is sensitive to high temperatures; therefore, heat can degrade chlorophylls.

Changes of chlorophyll contents in LJ during heating at 100°C for 30–150 min are shown in Table 2. Chlorophyll a, b and total chlorophyll contents gradually decreased with increasing heating time from 30 to 150 min. After heating for 150 min, contents of chlorophyll a and b were decreased about 45% and 27% compared to those of the control, respectively. According to Benlloch-Tinoco et al., structures of chlorophylls are sensitive to high temperatures, so heating degraded the chlorophyll a and b into pheophytins and pyropheophytins. Pheophytins were formed by the displacement of the central Mg$^{2+}$ atom from the chlorophyll phorphyrin ring with hydrogen. Under more extreme heating, the carbomethoxy group at the C10 of pheophytins was removed, and pyropheophytins were newly formed. In this study, we suspected that more chlorophylls were converted to pheophytins or pyropheophytins with longer heating times.

Color values

Changes of the color values ($L^*$, $a^*$, $b^*$, $\Delta E$, $H^*$, and $C^*$) of LJ after heating at 100°C with different heating time (30–150 min) are shown in Table 3. The $L^*$ values of LJ samples heated for 30–150 min were significantly decreased compared to those of the control, showing that LJ became dark after heating. Values of $a^*$ and $b^*$ of LJ were elevated with increasing heating time from 30 to 150 min; that is, the green component of color became less intense (increase in $a^*$) and the yellow component of color became more intense (increase in $b^*$). These results could be related to the degradation of chlorophylls in LJ, because chlorophylls were degraded into pheophytins and pyropheophytins under the high temperature, resulting in discoloration of LJ from bright green to yellowish-brown.

In general, a net color difference ($\Delta E$) higher than 1.8 is perceptible by the human eye and a value greater than 5.0 is regarded as a remarkable difference. As shown in Table 3, all values of $\Delta E$ of LJ samples heated for 30–150 min were higher than 1.8. These values were generally elevated with increasing heating time from 30 to 150 min. After heating for 120 and 150 min, the $\Delta E$ values were greater than 5.0 ($5.0 \pm 0.1$ and $5.7 \pm 0.2$, respectively), presenting a remarkable

Table 1. Effect of different heating times on pH and soluble solid contents of Lonicera japonica Thunb. at 100°C.

| Heating time (min) | pH     | Soluble solid contents (%) |
|-------------------|--------|-----------------------------|
| 0                 | 5.43 ± 0.01$^a$ | 5.7 ± 0.1$^a$               |
| 30                | 5.44 ± 0.01$^a$ | 5.8 ± 0.1$^a$               |
| 60                | 5.44 ± 0.01$^a$ | 5.7 ± 0.1$^a$               |
| 90                | 5.35 ± 0.02$^a$ | 5.7 ± 0.1$^a$               |
| 120               | 5.29 ± 0.02$^a$ | 5.8 ± 0.1$^a$               |
| 150               | 5.25 ± 0.00$^a$ | 5.8 ± 0.0$^a$               |

$^{a-d}$Values with different letters within the same column differ significantly (p<0.05).

Table 2. Effect of different heating times on chlorophyll content in Lonicera japonica Thunb. at 100°C.

| Heating time (min) | Chlorophyll a (ug/g) | Chlorophyll b (ug/g) | Total chlorophyll (ug/g) |
|-------------------|----------------------|----------------------|--------------------------|
| 0                 | 4.52 ± 0.04$^a$      | 2.4 ± 0.1$^a$        | 6.9 ± 0.1$^a$            |
| 30                | 3.49 ± 0.09$^b$      | 2.1 ± 0.1$^b$        | 5.7 ± 0.1$^b$            |
| 60                | 2.90 ± 0.03$^c$      | 2.0 ± 0.1$^c$        | 4.9 ± 0.1$^c$            |
| 90                | 2.58 ± 0.06$^d$      | 1.9 ± 0.0$^d$        | 4.4 ± 0.1$^d$            |
| 120               | 2.67 ± 0.06$^d$      | 1.8 ± 0.1$^d$        | 4.5 ± 0.1$^d$            |
| 150               | 2.48 ± 0.05$^e$      | 1.7 ± 0.0$^e$        | 4.2 ± 0.1$^e$            |

$^{a-c}$Values with different letters within the same column differ significantly (p<0.05).
color difference from the control. Therefore, heating for less than 90 min was desirable for retaining the original color of LJ.

Hue angle ($H^\circ$) is a qualitative indicator of the chromatic nature of the color. It is expressed in degrees (0° or 360° for red, 90°, 180°, and 270° for yellow, green, and blue, respectively). With increasing heating time from 30 to 150 min, the $H^\circ$ value of LJ was less than 90°. Chroma ($C^*$) value characterizes the quantitative attribute of colorfulness and is proportional to its intensity.\[24\]

The $C^*$ value of LJ significantly increased up to 60 min of heating. This marked effect on the color of LJ by heating for 30–150 min is related to the decrease in chlorophyll contents (Table 2), possibly because of the formation of pheophytins and pyropheophytins from the degradation of chlorophylls.\[23\]

**Total phenolic and total flavonoid contents**

Changes of total phenolic and total flavonoid contents in LJ during heating with different heating times (30–150 min) are given in Table 4. Total phenolic and total flavonoid contents in the control were 2013 ± 23 µg GAE/100 mg and 4495 ± 99 µg RE/100 mg, respectively. Compared to the control, significant increases in total phenolic and total flavonoid contents were found in the LJ samples heated for 30–60 min. In particular, the maximum contents of total phenolic (2594 ± 73 µg GAE/100 mg) and total flavonoid (5644 ± 57 µg RE/100 mg) contents in LJ were observed at 60 min of heating time. According to Uslu & Özcan,\[25\] total phenolic contents in the heat-treated sour cherry extract (160°C, 60 min) were increased compared to those in the non-heated one. In addition, Sharma et al.\[10\] reported that total flavonoid contents in onion were increased after heating (80–150°C, 30 min). Uslu & Özcan\[25\] proposed that the increases in total phenolic and total flavonoid contents after heating were caused by disruption of cell walls, resulting in conversion or releasing of bound phenolics into free forms. Furthermore, Papoutsis et al.\[26\] suggested that thermal dehydration can release the bound phenolic groups by breaking down the cell structures of the food matrix, because phenolic compounds were primarily present in bound forms linked to the cell walls. Therefore, we suggest in this study that the increases in total phenolic and total flavonoid contents in LJ samples heated for 30–60 min resulted from the

| Heating time (min) | $L^*$ | $a^*$ | $b^*$ | $\Delta E$ | $H^\circ$ | $C^*$ |
|-------------------|-------|-------|-------|-----------|---------|------|
| 0                 | 39 ± 1a | 2.6 ± 0.1f | 19.5 ± 0.2c | -         | 824 ± 0.1a | 19.6 ± 0.2c |
| 30                | 37 ± 1b | 4.0 ± 0.1e | 22.4 ± 0.2b | 3.6 ± 0.2d | 798 ± 0.2b | 22.7 ± 0.2b |
| 60                | 37 ± 0b | 4.6 ± 0.0d | 23.2 ± 0.1a | 4.5 ± 0.1c | 789 ± 0.1c | 23.6 ± 0.1a |
| 90                | 37 ± 1b | 5.0 ± 0.1c | 23.0 ± 0.3a | 4.8 ± 0.2bc | 779 ± 0.2d | 23.6 ± 0.3a |
| 120               | 37 ± 1b | 5.2 ± 0.1b | 23.3 ± 0.3a | 5.0 ± 0.1b | 776 ± 0.1d | 23.9 ± 0.3a |
| 150               | 36 ± 2b | 5.5 ± 0.1a | 23.4 ± 0.8a | 5.7 ± 0.2a | 768 ± 0.5a | 24.0 ± 0.8a |

*Values with different letters within the same column differ significantly (p<0.05).

Table 4. Effect of different heating times on total phenolic and total flavonoid contents in *Lonicera japonica* Thunb. at 100°C.

| Heating time (min) | Total phenolic contents (µg GAE/100 mg) | Total flavonoid contents (µg RE/100 mg) |
|--------------------|----------------------------------------|---------------------------------------|
| 0                  | 2013 ± 23a                             | 4495 ± 99d                            |
| 30                 | 2423 ± 66b                             | 5502 ± 39b                            |
| 60                 | 2594 ± 73a                             | 5644 ± 57a                            |
| 90                 | 2582 ± 11a                             | 5432 ± 52b                            |
| 120                | 2303 ± 80c                             | 4995 ± 73c                            |
| 150                | 2297 ± 30c                             | 4957 ± 53c                            |

*GAE: gallic acid equivalent.

RE: rutin equivalent.

*Values with different letters within the same column differ significantly (p<0.05).
disruption of cell walls during heating, subsequently leading to the conversion or release of bound phenolics into free forms.

In contrast, there decreasing trends of the total phenolic contents and total flavonoid contents in LJ after longer heating times of 120 and 90 min, respectively, although both contents were still significantly higher than those of the control. As previously described by Henríquez et al.,\textsuperscript{[27]} total phenolic contents in apple peel by-products were decreased by drum-drying (110°C for 120 sec and 140°C for 30 sec). Moreover, Sharma et al.\textsuperscript{[10]} reported that total flavonoid contents in onions were decreased after heating at 150°C for 30 min. Those authors suggested that the decreases in total phenolic and total flavonoid contents after heating are induced by too high a temperature. Accordingly, in this study, the reductions of the total phenolic and total flavonoid contents in LJ after heating (120 and 90 min, respectively) were induced by the long exposure periods and the low thermal stability of phenolics and flavonoids. Therefore, we think that heating at 100°C up to 150 min could improve the total phenolic and total flavonoid contents in LJ, but heating for 60 min was the best treatment for increasing total phenolic and total flavonoid contents in LJ.

Quantification of individual phenolic acids using RP-HPLC

The effects of different heating time (30–150 min) on changes in the contents of the four phenolic acids in LJ were identified and quantified by RP-HPLC (Table 5). Chlorogenic acid, the ester of caffeic acid and quinic acid, is the major component in LJ.\textsuperscript{[28]} It has been reported that it has antioxidant activities in vitro by scavenging reactive oxygen species,\textsuperscript{[29]} antidiabetic activities by suppression of hepatic gluconeogenesis,\textsuperscript{[30]} and anti-obesity activities in vivo by reducing preadipocyte population growth.\textsuperscript{[31]}

In this study, chlorogenic acid in LJ was significantly increased by up to 60 min of heating. The chlorogenic acid content in the control was 1007 ± 37 µg/100 mg. After heating for 60 min, the chlorogenic acid content (1367 ± 2 µg/100 mg) was significantly increased and was retained up to 90 min, perhaps for the following reasons. First, the increases in chlorogenic acid contents in LJ samples heated for 30–90 min could result from the destruction of cell walls during heating. According to Acosta-Estrada et al.,\textsuperscript{[9]} chlorogenic acid in foods forms ester linkages with carbohydrates and proteins through their carboxyl groups and ether linkages with lignin through their hydroxyl groups. They reported that heat can break down the ester and ether linkages between bound phenolic groups and other food matrixes, consequently releasing bound chlorogenic acid into free forms.

Second, the increases in chlorogenic acid contents in LJ after heating for 30–90 min could result from the hydrolysis of dicafeoylquinic acid into chlorogenic acid. Clifford et al.\textsuperscript{[32]} reported that the amounts of chlorogenic acid doubled after 5 min of roasting time because of the partial hydrolysis of dicafeoylquinic acid such as 3,5-dicafeoylquinic acid and 4,5-dicafeoylquinic acid to chlorogenic acid. Accordingly, we think the chlorogenic acid contents in LJ samples heated for 30–90 min were increased by the release of chlorogenic acids by the destruction of cell walls and the hydrolysis of dicafeoylquinic acid to chlorogenic acid.

| Heating time (min) | Phenolic acids contents (µg/100 mg) | Flavonoids contents (µg/100 mg) |
|--------------------|-----------------------------------|-------------------------------|
|                    | Chlorogenic acid | Caffeic acid | 4,5-dicafeoylquinic acid | 3,5-dicafeoylquinic acid | Rutin | Quercetin | Luteolin |
| 0                  | 1007 ± 37\textsuperscript{d}  | 1.9 ± 0.1\textsuperscript{d} | 13.4 ± 0.4\textsuperscript{d} | 649 ± 21\textsuperscript{c} | 36 ± 3\textsuperscript{bc} | 6.5 ± 0.3\textsuperscript{bc} | 0.09 ± 0.02\textsuperscript{d} |
| 30                 | 1261 ± 11\textsuperscript{b} | 2.6 ± 0.0\textsuperscript{b} | 18.9 ± 0.6\textsuperscript{b} | 800 ± 2\textsuperscript{a} | 42 ± 0\textsuperscript{a} | 7.3 ± 0.3\textsuperscript{b} | 0.15 ± 0.02\textsuperscript{c} |
| 60                 | 1367 ± 2\textsuperscript{a} | 3.0 ± 0.0\textsuperscript{a} | 25.3 ± 0.3\textsuperscript{a} | 791 ± 1\textsuperscript{a} | 42 ± 1\textsuperscript{a} | 8.1 ± 0.1\textsuperscript{a} | 0.16 ± 0.03\textsuperscript{bc} |
| 90                 | 1390 ± 30\textsuperscript{a} | 3.0 ± 0.1\textsuperscript{a} | 30.3 ± 0.3\textsuperscript{a} | 740 ± 20\textsuperscript{b} | 41 ± 1\textsuperscript{a} | 8.4 ± 0.2\textsuperscript{c} | 0.21 ± 0.02\textsuperscript{a} |
| 120                | 1215 ± 26\textsuperscript{c} | 2.4 ± 0.1\textsuperscript{c} | 28.5 ± 0.5\textsuperscript{b} | 614 ± 14\textsuperscript{d} | 37 ± 0\textsuperscript{b} | 7.1 ± 0.4\textsuperscript{b} | 0.20 ± 0.01\textsuperscript{ab} |
| 150                | 1030 ± 12\textsuperscript{d} | 2.0 ± 0.1\textsuperscript{d} | 26.8 ± 0.6\textsuperscript{d} | 462 ± 8\textsuperscript{c} | 31 ± 0\textsuperscript{c} | 5.6 ± 0.4\textsuperscript{d} | 0.10 ± 0.03\textsuperscript{d} |

\textsuperscript{a-f} Values with different letters within the same column differ significantly (p<0.05).
However, when the heating time was over 120 min, chlorogenic acid in LJ began to significantly decrease. We can suggest four mechanisms on for these. First, the acyl migration could induce the reductions of chlorogenic acid in LJ during heating for 120–150 min. Chlorogenic acid is referred to 5-caffeoylquinic acid according to IUPAC numbering, and it has isomers, such as neochlorogenic acid (3-caffeoylquinic acid) and cryptochlorogenic acid (4-caffeoylquinic acid). According to Liang et al., the chlorogenic acid in coffee was significantly decreased after roasting at 210°C for 12 min, whereas the levels of neochlorogenic acid and cryptochlorogenic acid were significantly increased. These authors mentioned that the reason for the decreases in chlorogenic acid and increases in neochlorogenic acid and cryptochlorogenic acid after roasting was acyl migration via the formation of an ortho-ester intermediate. Acyl migration took place by the heating before dehydration at the quinic acid moiety (lactonization), which moved the 5-acyl group in chlorogenic acid to position C3 (neochlorogenic acid) and C4 (cryptochlorogenic acid).

Second, the decreases in chlorogenic acid content in LJ during heating for 120–150 min could be caused by lactonization of chlorogenic acid. Jeon et al. explained that the content of chlorogenic acid in medium roasted coffee was about 3–5 times higher than that in medium-dark roasted coffee, regardless of the coffee’s country of origin. They showed that chlorogenic acid was dehydrated during the roasting, thereby producing the corresponding lactones by the loss of a water molecule from the quinic acid moiety. Moreira et al. also mentioned that chlorogenic acid newly generated an intramolecular ester bond in quinic acid between C1 and C5, mainly by losing a water molecule from the quinic acid moiety, consequently converting into chlorogenic acid lactones (3-caffeoylquinic acid lactone and 4-caffeoylquinic acid lactone).

Third, hydrolysis could be why chlorogenic acid is reduced in LJ after heating for 120–150 min. Moreira et al. showed that chlorogenic acid was hydrolyzed to the phenolic moiety (caffeic acid) and non-phenolic moiety (quinic acid) by roasting. Moisture content (6.06%) in fresh LJ and released moisture by lactonization could act as a substrate for hydrolysis of chlorogenic acid to caffeic acid and quinic acid during heating.

Last, thermal degradation (pyrolysis) could be the reason for this decrease in chlorogenic acid in LJ. According to Moreira et al., upon heating, chlorogenic acid resulted in decarboxylation forming simple phenols, such as pyrogallol, hydroxyhydroquinone, catechol, 4-ethylcatechol, and 4-methylcatechol. These authors said that the caffeic acid moiety of chlorogenic acid changed into catechol, 4-ethylcatechol, and 4-methylcatechol, and the quinic acid moiety of chlorogenic acid formed pyrogallol, hydroxyhydroquinone, and catechol during heating, subsequently causing the decreases in chlorogenic acid.

The amount of caffeic acid in LJ was significantly increased during 60 min of heating and was then retained up to 90 min, representing about a 54.2% increase over that in the control (Table 5). Del Pino-García et al. reported that the increases in caffeic acid in powdered red-wine pomace seasonings were observed after heating (90°C, 90 min) and suggested that this could be caused by release of caffeic acid bound to the food matrixes and by partial degradation of lignin, which liberated caffeic acid. Moreover, they also said that heating could break esterified forms of hydroxycinnamic acids, such as chlorogenic acid, leading to the increase in caffeic acid content. Therefore, in this study, the increases in caffeic acid in LJ samples heated for 30–90 min could have resulted from the the conversion of bound caffeic acids into free forms and hydrolysis of chlorogenic acid to caffeic acid.

In contrast, the amount of caffeic acid in LJ samples heated for more than 120 min began to decrease significantly. According to Dawidowicz & Typek, when coffee was roasted at 170–230°C for 10–30 min, caffeic acid was not found, because of its low thermal stability. Accordingly, we think that caffeic acid in LJ was degraded by 120 min of heating because the long exposure reduced the caffeic acid content in LJ.

Changes in the contents of the other phenolic acids (4,5-dicaffeoylquinic acid and 3,5-dicaffeoylquinic acid) in LJ after heating are shown in Table 5. The contents of 4,5-dicaffeoylquinic acid and
3,5-dicaffeoylquinic acid in LJ were significantly increased by 90 and 30 min of heating time, respectively. The 4,5-dicaffeoylquinic acid content in LJ heated for 90 min was about 2.26 times that of the control, and 3,5-dicaffeoylquinic acid content in LJ heated for 30 min was about 1.23 times that of the control. In general, isochlorogenic acids, such as 3,5-dicaffeoylquinic acid and 4,5-dicaffeoylquinic acid, are covalently bound to structural carbohydrates, protein, and lignin. Heating broke the linkages between isochlorogenic acids and cell walls, and consequently bound compounds became free forms. Therefore, in this study, the increases in 4,5-dicaffeoylquinic acid in LJ samples heated for 30–90 min and in 3,5-dicaffeoylquinic acid in LJ heated for 30 min could result from the transformation of bound isochlorogenic acids into free forms.

However, the contents of 4,5-dicaffeoylquinic acid and 3,5-dicaffeoylquinic acid in LJ were significantly decreased by 120 and 90 min of heating, respectively. Especially, the 3,5-dicaffeoylquinic acid content in LJ heated for 150 min was significantly lower than that of the control, with a retention rate of 71.2%, although 4,5-dicaffeoylquinic acid content in the control was doubled after 150 min of heating. According to Li et al., the order of thermal stability was 4,5-dicaffeoylquinic acid >3,5-dicaffeoylquinic acid. Therefore, in this study, 3,5-dicaffeoylquinic acid in LJ was thermally degraded more than was the 4,5-dicaffeoylquinic acid in LJ during heating.

**Quantification of individual flavonoids using RP-HPLC**

The effect of different heating times (30–150 min) on changes in the contents of three flavonoids (rutin, quercetin, and luteolin) in LJ were identified and quantified by RP-HPLC (Table 5). In this study, the contents of rutin, quercetin, and luteolin in LJ began to increase by heating after 30 min. The contents of rutin, quercetin, and luteolin in the control were 36 ± 3, 6.5 ± 0.3, and 0.09 ± 0.02 ug/100 mg, respectively. The increases in rutin, quercetin, and luteolin in LJ were observed during 30, 60, and 90 min of heating time, respectively. According to Li et al., when orange by-products were dried at 100°C for 48 h, the contents of rutin and luteolin were significantly increased. Furthermore, Júaniz et al. reported that quercetin and luteolin in green pepper were significantly increased after heating twice at 150°C for 10 min and then 110°C for 5 min; they reported that heating softened the structures of vegetables by cell ruptures, leading to conversion of bound quercetin and luteolin into free forms. Therefore, we think the increases in rutin, quercetin, and luteolin in LJ during heating for 30, 60, and 90 min, respectively, were induced by conversion of those bound compounds into free compounds.

In contrast, both rutin and quercetin in LJ began to decrease significantly during 120 min of heating time. In particular, the contents of rutin and quercetin in LJ heated for 150 min were significantly lower than those of the control. The decreases in rutin and quercetin in LJ samples heated for 120–150 min were consistent with Chaaban et al. who reported that rutin was degraded by heating from 70 to 130°C for 120 min. In addition, Sharma et al. found that six onion varieties showed decreases in flavonoid contents, especially quercetin, after heating at 150°C for 30 min.

**Quantification of individual organic acids using HPLC**

The changes of contents of individual organic acids (quinic acid, malic acid, shikimic acid, citric acid, and succinic acid) in LJ during different heating times (30–150 min) were quantified by HPLC, as shown in Table 6. In this study, quinic acid was the major organic acid in LJ. As heating time passed from 30 to 150 min, quinic acid significantly increased from 2488 ± 4 to 2777 ± 15 ug/100 mg. Increases in quinic acid in LJ during heat treatments at 100°C for 30–150 min could result from the hydrolysis of chlorogenic acid and isochlorogenic acids into quinic acid. According to Moreira et al., coffee roasting promoted hydrolysis of chlorogenic acid and isochlorogenic acid, thus forming quinic acid.

Shikimic acid content in LJ was significantly increased by 150 min of heating. Under high temperature, quinic acid could be transformed into quinic acid lactone or shikimic acid. Jaiswal
et al.\textsuperscript{[43]} showed that shikimic acid was formed by dehydration of quinic acid, losing 1-OH functionality. Deshpande et al.\textsuperscript{[35]} found that the conversion rate of quinic acid into shikimic acid was less than that of lactonization of quinic acid. Therefore, the significant but slight increments of the contents of shikimic acid in LJ during heating for 30–150 min could result from the transformation of quinic acid to shikimic acid.

**Assessment of antioxidant properties**

Antioxidant properties of LJ samples heated for 30–150 min were evaluated for DPPH radical scavenging activity, ABTS radical scavenging activity, FRAP value, and reducing power, and the results are given in Figure 1. DPPH radical scavenging activity and ABTS radical scavenging activity reflect the ability of hydrogen-donating antioxidants and electron transfer to scavenge DPPH and ABTS\textsuperscript{+} radicals. In this study, findings on DPPH and ABTS radical scavenging activities of LJ during heating for 30–150 min showed similar tendencies. DPPH and ABTS radical scavenging activity of LJ were significantly increased during 60 min of heating and were retained for 90 min. In particular, after 60 min of heating, DPPH and ABTS radical scavenging activities of LJ were significantly improved by about 29 and 35%, respectively.

FRAP and reducing power assays represent their ability to reduce the ferric (Fe\textsuperscript{3+}) form to the ferrous (Fe\textsuperscript{2+}) form. In the FRAP assay, the reaction of an antioxidant compound with a Fe\textsuperscript{3+}-TPTZ complex formed a blue-colored Fe\textsuperscript{2+}-TPTZ. Similarly, in the reducing power assay, a Prussian blue-colored complex was formed by adding FeCl\textsubscript{3} to the Fe\textsuperscript{2+} form, and consequently reduction can be evaluated. We observed that the FRAP values and reducing powers of LJ were significantly increased by heating for 60 min.

The increasing trends of antioxidant properties of LJ after heating for 60 min corresponded to the results reported by Kao et al.\textsuperscript{[44]} and De Santiago et al.\textsuperscript{[45]} Kao et al.\textsuperscript{[44]} reported that Trolox equivalent antioxidant capacities of cilantro and sweet potato leaf were increased after boiling for 1 min. Also, De Santiago et al.\textsuperscript{[45]} reported that antioxidant properties of cactus cladodes were significantly improved after griddling (150°C, 5 min and then 110°C, 5 min). These authors proposed that the antioxidant properties were increased by the breakage of linkages between bound phenolics and cell walls by thermal hydrolysis of cell walls and subcellular compartments, leading to increases in free phenolics contents. Therefore, the improvement of antioxidant properties of LJ during heating for 60 min could result from the increase in free phenolic contents related to antioxidant properties of LJ.

When the heating time was over 120 min, we observed that antioxidant properties of LJ were significantly decreased. Nevertheless, DPPH radical scavenging activities, ABTS radical scavenging activities, FRAP values, and reducing powers of LJ samples heated for 120 and 150 min were significantly still higher than those of the control. According to Oancea et al.\textsuperscript{[46]} 10% and 17% losses of DPPH radical scavenging activities were observed in sour-cherry extracts heated for 60 min at 100 and 150°C, respectively. They reported that DPPH radical scavenging activity declined during heating because phenolic or other compounds responsible for antioxidant capacity were degraded. Therefore, the declines of antioxidant properties of LJ after heating for 120 min resulted because the

| Heating time (min) | Quinic acid | Malic acid | Shikimic acid | Citric acid | Succinic acid |
|--------------------|-------------|------------|---------------|-------------|--------------|
| 0                  | 2488 ± 4\textsuperscript{d} | 511 ± 15\textsuperscript{ab} | 15.4 ± 0.3\textsuperscript{d} | 170 ± 10\textsuperscript{f} | 663 ± 8\textsuperscript{ab} |
| 30                 | 2563 ± 8\textsuperscript{a} | 515 ± 7\textsuperscript{a} | 15.5 ± 0.1\textsuperscript{d} | 231 ± 16\textsuperscript{g} | 617 ± 16\textsuperscript{c} |
| 60                 | 2676 ± 26\textsuperscript{b} | 480 ± 18\textsuperscript{f} | 16.1 ± 0.1\textsuperscript{c} | 543 ± 20\textsuperscript{d} | 592 ± 60\textsuperscript{c} |
| 90                 | 2699 ± 23\textsuperscript{b} | 484 ± 3\textsuperscript{a} | 16.2 ± 0.4\textsuperscript{c} | 754 ± 13\textsuperscript{c} | 670 ± 15\textsuperscript{b} |
| 120                | 2704 ± 5\textsuperscript{a} | 489 ± 13\textsuperscript{bc} | 17.3 ± 0.2\textsuperscript{b} | 855 ± 14\textsuperscript{b} | 685 ± 29\textsuperscript{a} |
| 150                | 2777 ± 15\textsuperscript{a} | 478 ± 18\textsuperscript{a} | 18.1 ± 0.1\textsuperscript{a} | 956 ± 22\textsuperscript{a} | 630 ± 4\textsuperscript{bc} |

\textsuperscript{a-f}Values with different letters within the same column differ significantly (p<0.05).
heating reduced the contents of phenolic compounds relevant to antioxidant capacities (Table 5). In conclusion, heating at 100°C for 60 min was the optimal treatment for improving antioxidant properties of LJ.

**Pearson correlations**

Pearson correlation coefficients between nutraceuticals (total phenolics, total flavonoids, chlorogenic acid, caffeic acid, 4,5-dicaffeoylquinic acid, 3,5-dicaffeoylquinic acid, rutin, quercetin, and luteolin) and antioxidant properties (DPPH and ABTS radical scavenging activity, FRAP value, and reducing power) of LJ are shown in Table 7. Total phenolic content in LJ was positively correlated with the DPPH radical scavenging activity \( (r = 0.99, p < 0.01) \), ABTS radical scavenging activity \( (r = 0.96, p < 0.01) \), FRAP value \( (r = 0.96, p < 0.01) \), and reducing power \( (r = 0.99, p < 0.001) \). Also, total flavonoid content in LJ was positively correlated with the DPPH radical scavenging activity \( (r = 0.94, p < 0.01) \), ABTS radical scavenging activity \( (r = 0.86, p < 0.05) \), FRAP value \( (r = 0.91, p < 0.05) \), and reducing power \( (r = 0.95, p < 0.01) \). These findings were consistent with Foo et al.\(^{47}\) and Lin et al.\(^{48}\) who reported that total phenolic and total flavonoid contents had high correlation coefficient with antioxidant properties such as DPPH and ABTS radical scavenging activity, FRAP value, and reducing power \( (p < 0.05) \).

Individual phenolic acids (chlorogenic acid and caffeic acid) and flavonoids (quercetin) in LJ were positively related to the antioxidant properties of LJ. Chlorogenic acid and caffeic acid in LJ were positively correlated with DPPH radical scavenging activity \( (r = 0.94–0.96, p < 0.01) \) and

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*Figure 1. Effect of different heating times on antioxidant properties of *Lonicera japonica* Thunb. at 100°C. Different letters on the bars indicate significant differences \( (p < 0.05) \).*
ABTS radical scavenging activity \( (r = 0.88-0.89, \ p < 0.05) \), and quercetin content in LJ was also positively correlated with DPPH radical scavenging activity \( (r = 0.83, \ p < 0.05) \). According to Miao et al.\(^{49}\) the contents of chlorogenic acid, caffeic acid, and quercetin were negatively correlated with EC\(_{50}\) value of DPPH radical scavenging activity \( (p < 0.01) \). Also, Aires et al.\(^{50}\) showed that chlorogenic acid and caffeic acid were positively correlated with antioxidant activity \( (p < 0.001) \). In general, the antioxidant properties of phenolic acids depend on the position and number of hydroxyl groups linked to the aromatic ring. According to Kono et al.\(^{51}\) chlorogenic acid and caffeic acid can donate an electron to stabilize nitrogen-centered free DPPH radicals. Wu\(^{52}\) also reported that chlorogenic acid can scavenge the DPPH radicals. Furthermore, chlorogenic acid and caffeic acid in LJ had positive correlations with FRAP value \( (r = 0.94-0.97, \ p < 0.01) \) and reducing power \( (r = 0.95, \ p < 0.01) \), and quercetin content in LJ was also significantly related to FRAP value \( (r = 0.87, \ p < 0.05) \) and reducing power \( (r = 0.82, \ p < 0.05) \). Miao et al.\(^{49}\) showed that the contents of chlorogenic acid, caffeic acid, and quercetin were positively correlated with antioxidant activity \( (p < 0.001) \). In general, the antioxidant properties of phenolic acids depend on the position and number of hydroxyl groups linked to the aromatic ring. According to Kono et al.\(^{51}\) chlorogenic acid and caffeic acid can donate an electron to stabilize nitrogen-centered free DPPH radicals. Wu\(^{52}\) also reported that chlorogenic acid can scavenge the DPPH radicals. Furthermore, chlorogenic acid and caffeic acid in LJ had positive correlations with FRAP value \( (r = 0.94-0.97, \ p < 0.01) \) and reducing power \( (r = 0.95, \ p < 0.01) \), and quercetin content in LJ was also significantly related to FRAP value \( (r = 0.87, \ p < 0.05) \) and reducing power \( (r = 0.82, \ p < 0.05) \). Miao et al.\(^{49}\) showed that the contents of chlorogenic acid, caffeic acid, and quercetin were positively correlated with FRAP value \( (p < 0.01) \), and Lee et al.\(^{53}\) demonstrated that quercetin content was positively correlated with FRAP value \( (p < 0.01) \). According to Wu,\(^{52}\) chlorogenic acid reduced Fe\(^{3+}\) to Fe\(^{2+}\), and Firuzi et al.\(^{54}\) reported that quercetin could effectively reduce Fe\(^{3+}\) ions. Therefore, we found that the total phenolic and total flavonoid contents in LJ had strongly positive correlations with antioxidant properties. In particular, chlorogenic acid, caffeic acid, and quercetin contents significantly influenced the antioxidant properties of LJ, and those compounds were the main active compounds in LJ.

**Conclusion**

In this study, heating at 100°C with different heating times \( (30–150 \text{ min}) \) were applied to LJ. According to the changes in chlorophyll contents in LJ after heating for 30–150 min, the decrease of chlorophyll of LJ. The total phenolic and total flavonoid contents in LJ, which were relevant to antioxidant properties, were significantly improved by 60 min of heating, so heating at 100°C for 60 min was the best treatment for improving total phenolic and total flavonoid contents in LJ. The analysis of individual phenolic acids (chlorogenic acid, caffeic acid, 4,5-dicaffeoylquinic acid, and 3,5-dicaffeoylquinic acid) and flavonoids (rutin, quercetin, and luteolin) in LJ by RP-HPLC revealed that the contents of individual phenolic acids and flavonoids were significantly increased during heating. In particular, 60 min of heating was adequate for improving the contents of chlorogenic acid and caffeic acid.

| Variables | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 |
|-----------|---|---|---|---|---|---|---|---|---|----|----|----|----|
| 1. Total phenolic | 1 | | | | | | | | | | | | |
| 2. Total flavonoid | 0.95** | 1 | | | | | | | | | | | |
| 3. Chlorogenic acid | 0.91* | 0.89* | 1 | | | | | | | | | | |
| 4. Caffeic acid | 0.93** | 0.91* | 0.99*** | 1 | | | | | | | | | |
| 5. 4,5-dicaffeoylquinic acid | 0.66 | 0.46 | 0.52 | 0.48 | 1 | | | | | | | | |
| 6. 3,5-dicaffeoylquinic acid | 0.54 | 0.67 | 0.76 | 0.78 | −0.16 | 1 | | | | | | | |
| 7. Rutin | 0.63 | 0.74 | 0.84* | 0.85* | −0.02 | 0.99*** | 1 | | | | | | |
| 8. Quercetin | 0.75 | 0.72 | 0.94** | 0.94** | 0.34 | 0.83 | 0.88* | 1 | | | | | |
| 9. Luteolin | 0.68 | 0.59 | 0.83* | 0.77 | 0.70 | 0.45 | 0.56 | 0.78 | 1 | | | | |
| 10. DPPH radical scavenging activity | 0.99** | 0.94** | 0.94** | 0.96** | 0.60 | 0.63 | 0.71 | 0.83* | 0.69 | 1 | | | |
| 11. ABTS radical scavenging activity | 0.96** | 0.86* | 0.88* | 0.89* | 0.74 | 0.45 | 0.54 | 0.75 | 0.68 | 0.96** | 1 | | |
| 12. FRAP | 0.96** | 0.91* | 0.94** | 0.97** | 0.54 | 0.67 | 0.74 | 0.87* | 0.66 | 0.99*** | 0.95** | 1 |
| 13. Reducing power | 0.99*** | 0.95** | 0.95** | 0.96** | 0.63 | 0.61 | 0.70 | 0.82* | 0.71 | 1*** | 0.96** | 0.98*** | 1 |

*Only significantly correlated variables and coefficients are shown.

\(^*p < 0.05; **p < 0.01; ***p < 0.001.\)
acid, caffeic acid, and quercetin in LJ. The destruction of cell structures of LJ by heating made bound phenolic compounds into free forms, resulting in increases of phenolic compounds. Based on the results of antioxidant properties (DPPH and ABTS radical scavenging activity, FRAP value, and reducing power), we also observed that 60 min of heating was enough to elevate antioxidant properties of LJ. Since phenolic compounds can function as antioxidants, total phenolic, total flavonoid, chlorogenic acid, caffeic acid, and quercetin contents were positively correlated with the antioxidant properties of LJ. Consequently, these results suggest that heating at 100°C for a moderate duration (60 min in this study) can be used in LJ processing, improving several nutraceutical and antioxidant properties of LJ.

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