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Spent coffee as a rich source of antioxidative compounds

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Abstract Antioxidant activities and major antioxidants were investigated in the methanolic extracts of roasted and spent coffee to evaluate the feasibility of spent coffee as a source of functional ingredients. Phenolic compounds, such as gallic acid, protocatechuic acid, and chlorogenic acid, and nitrogenous compounds, including trigonelline and caffeine, were identified. Caffeine was the most abundant compound, followed by chlorogenic acid. Despite the significant reduction of antioxidants, 2,2,-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity was retained in more than 95% of roasted coffee. The retentions of superoxide dismutase (SOD)-like activity and ferric reducing antioxidant power (FRAP) were 65–90 and 46–60%, respectively. Gallic acid had a positive correlation with SOD-like activity, whereas protocatechuic acid positively correlated with FRAP, suggesting that the major compounds contributing to each antioxidant activity are different. These results show that spent coffee can be used as an antioxidant source for functional foods and cosmetic products to improve antioxidant properties.

Keywords Spent coffee · Caffeine · Chlorogenic acid · DPPH · SOD-like · FRAP

Introduction

Coffee is one of the most widely consumed beverages in Korea. According to the latest figures from the International Coffee Organization [1], coffee consumption in South Korea amounted to 120,000 tons in 2015. This produced an estimated 96,000 tons of solid residue of coffee, called spent coffee, which is generated during the brewing of coffee. The disposal of spent coffee causes environmental pollution. This has led to the substance being reused as animal feed, fertilizers, biodiesel, and sorbent for metal removal [2–5].

Roasted coffee contains many antioxidants such as phenolic compounds, caffeine, trigonelline, and Maillard reaction products [6]. Of the polyphenols present in coffee, chlorogenic and gallic acid efficiently eliminate the reactive oxygen species induced by glutathione depletion and lipid peroxidation [7]. In addition, caffeine and trigonelline show antiradical activities in vitro and in vivo [8]. Fractions of Maillard reaction products in coffee also contribute to the antioxidant activity [9]. Earlier studies focused on the effects of the degree to which the coffee was roasted on its antioxidant capacity [9–11]. A considerable amount of antioxidants remains in the spent coffee after brewing [12]. Recently, more attention has been paid to recycling spent coffee for the development of healthier products.

Therefore, this study aimed at evaluating the feasibility of spent coffee as a valuable source of antioxidants by comparing antioxidant activities and the major antioxidative compounds in roasted and spent coffee. The spent and roasted coffee may differ in coffee variety, origin, roasting condition, and brewing method.

Materials and methods

Sample material and chemicals

Three samples of spent coffee generated from espresso extraction of roasted coffee at 92–95 °C for 15–20 s were

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supplied from local cafes in Seoul, Korea. The variety of two of the samples (AR-1 and AR-2) was *Coffee arabica*, while the third sample (BL) was a blend comprising various coffee species. DPPH, 2,4,6-tri-(2-pyridyl)-1,3,5-triazine (TPTZ), xanthine, xanthine oxidase (from bovine milk), nitroblue tetrazolium (NBT), ethylenediaminetetraacetic acid (EDTA), Folin–Ciocalteu's phenol reagent, methanol, ethanol, potassium iodate (KIO_3), potassium persulfate ($K_2S_2O_8$), monosodium phosphate (NaH_2PO_4), disodium phosphate (Na_2HPO_4), sodium bicarbonate ($NaHCO_3$), hydrogen chloride (HCl), ferric chloride ($FeCl_3$), ferrous sulfate ($FeSO_4$), ascorbic acid, α-tocopherol, formic acid, acetonitrile, tannic acid, trigonelline, gallic acid, protocatechuic acid, chlorogenic acid, and caffeine were purchased from Sigma-Aldrich (St Louis, MO, USA).

Sample extraction

The roasted and spent coffee samples were dried in an oven at 105 °C for 24 h. The extraction was based on a method optimized by Mussatto et al. [2]. The dried sample (1 g) was mixed with 40 mL of 60% methanol and shaken at 120 rpm for 90 min at 60 °C. The mixture was then centrifuged at 16,582×*g* for 10 min at 4 °C. The supernatant was filtered through a 0.45 μm Millex-FH hydrophobic fluoropure (PTFE) membrane (Millipore, Milford, MA, USA) and stored at −35 °C in darkness until analyzed.

Determination of total tannins

The total tannin content was determined using a potassium iodate test described by Naima et al. [13]. Standard solutions of tannic acid for calibration were prepared in concentrations of 0, 100, 500, 1000, and 2500 μg/mL in distilled water. Five mL of 2.5% KIO_3 solution, preheated for 7 min at 30 °C, was mixed with 1 mL of tenfold diluted extracts. The mixture was then placed in a thermostatic bath at 30 °C for 2 min, and absorbance was measured at 550 nm (Biochrom Libra S22, Santa Barbara, CA, USA). The total tannin content was expressed as mg tannic acid equivalent (TAE) per g dry weight. All samples were analyzed in triplicate.

Determination of total phenolic content

The total phenolic content was measured by deploying a colorimetric method described by Singleton and Rossi [14]. First, a tenfold diluted extract (0.5 mL) was mixed with 0.9 mL of tenfold diluted Folin–Ciocalteu reagent and 3.6 mL of saturated sodium bicarbonate solution. The mixture was then placed for 1 h at room temperature, and absorbance was measured at 700 nm. The total phenolic content was expressed as mg of gallic acid equivalents (GAE) per g dry weight. All samples were analyzed in triplicate.

Qualification of antioxidative compounds

The extracts of roasted and spent coffee were analyzed to identify antioxidative compounds using a Waters® ACQUITY™ Ultra-Performance Liquid Chromatograph (UPLC) coupled with a diode array detector and a quadrupole-time of flight-mass spectrometer (qTOF-MS, Waters, Milford, MA, USA). The reverse-phase column (CORTECS™ UPLC® C_{18} 1.6 μm, 50 mm × 2.1) was used for chromatographic separations at 40 °C. The injection volume was 5 μL, and the flow rate was 300 μL/min. The mobile phase consisted of 0.1% formic acid (solvent A) and acetonitrile (solvent B). The gradient was as follows: 0–3 min, 1–5% B; 3–6 min, 5–10% B; 6–9 min, 10–15% B; 9–15 min, 15–60% B. Mass spectral data were collected in the positive mode using an electrospray ionization (ESI) source. A source temperature of 120 °C, desolvation temperature of 350 °C, and capillary voltage of 30 kV were applied. Argon was used as the collision gas at a flow rate of 100 L/h and nitrogen as the desolvation gas at a flow rate of 800 L/h.

Quantification of antioxidative compounds

Antioxidative compounds were analyzed using a high performance liquid chromatograph (HPLC) equipped with a diode array detector (Agilent Technologies, 1260 Infinity, Waldbronn, Germany). A Kinetex 5 μm C_{18} column (150 mm × 4.6 mm i.d.; Phenomenex, Torrance, CA, USA) was employed at 40 °C. The injection volume was 4 μL. The antioxidative compounds were eluted using a gradient mobile phase consisting of 0.1% formic acid (solvent A) and acetonitrile (solvent B) at a flow rate of 1 mL/min. The gradient was programmed as follows: 0–10 min, 15–37% B; 5–10 min, 37–80% B; 10–12 min, 80–100%, B; 12–13 min, 100–15% B. The separated compounds were monitored at 258 nm. The identity of the major antioxidative compounds was based on the congruence of the retention times and UV–visible spectra with that of pure authentic standards (trigonelline, gallic acid, protocatechuic acid, chlorogenic acid, and caffeine).

Antioxidant activity determination

The DPPH radical scavenging activity was determined using a method described by Xu et al. [15] with some modifications. Each extract (1 mL) was mixed with 2 mL of 0.2 mmol/L DPPH solution, which was vigorously shaken and allowed to stand at room temperature for 30 min in the dark. DPPH radical scavenging activity was calculated as the percentage decrease of the absorbance at

517 nm relative to a blank (as 100%). The positive control was 0.05% gallic acid, 0.05% ascorbic acid, and 0.05% α-tocopherol. The concentrations of positive control were selected based on the content of gallic acid present in the spent coffee extract (0.01–0.05%). The percentage of the scavenging activity of the DPPH radical was calculated using the following equation:

$$\text{DPPH radical scavenging activity}(\%) = (A_{blank} - A_{sample}/A_{blank}) \times 100.$$

In this equation, A_{blank} and A_{sample} are the absorbances of the control and extract, respectively. All samples were analyzed in triplicate.

The ferric reducing antioxidant power (FRAP) assay was determined using the redox-linked colorimetric method of Ranic et al. [16] with slight modifications. A FRAP reagent was freshly prepared using 300 mmol/L acetate buffer (pH 3.6), 20 mmol/L ferric chloride, and 10 mmol/L tripyridyltriazine in 40 mmol/L HCl. These three solutions were mixed together at a ratio of 10:1:1 (v/v/v). A ferrous sulfate solution was then prepared at 1000 µmol/L in distilled water. An aliquot of 0.1 mL of working solutions (0, 100, 200, 300, 400, 500, 700, 900, and 1000 µmol/L) were mixed with 3 mL of the FRAP reagent and incubated at 37 °C for 30 min before absorbance was measured at 593 nm. The extract (0.1 mL) was analyzed as described above. The antioxidant capacity, based on the ability to reduce ferric ions in the sample, was calculated from the linear calibration curve and expressed as mmol $FeSO_4$ equivalents per mL of sample. All samples were analyzed in triplicate.

The superoxide dismutase (SOD)-like activity was determined using the method described by Koh and Surh [17] with some modifications. An aliquot of 0.1 mL of each extract was mixed with 2.8 mL reducing solution (250 µmol/L xanthine in 0.1 mmol/L EDTA phosphate buffer pH 7.4 and NBT). Xanthine oxidase (0.2 mL, 0.1 unit/mL) was added and then incubated at 37 °C for 40 min. Absorbance was measured at 550 nm. The radical scavenging activity was calculated as the percentage inhibition of the reduced NBT relative to the blank (as 100%). The positive controls of 0.05% gallic acid, 0.05% ascorbic acid, and 0.05% α-tocopherol were used for comparison. The scavenging activity was calculated using the equation described for DPPH.

Statistical analysis

All statistical analyses were performed using the SPSS IBM version 21.0 (Statistical Package for Social Science, SPSS Inc., Chicago, IL, USA). The average and standard deviations were calculated from triplicate measurements. A one-way analysis of variance (ANOVA) was used to determine the significant differences between samples. Differences between roasted and spent coffee were analyzed for statistical significance using Student's *t*-test. Associations among antioxidative compound levels and activities in coffee were determined by using Pearson's correlation analysis.

Results and discussion

Total tannin and total phenolic content

The total tannin content in roasted coffee ranged between 0.7 and 0.9 mg TAE/g (Fig. 1). Hecimovic et al. [18] demonstrated that tannin content in roasted coffee varies depending on the variety and degree of roasting. The total tannin content was significantly lower in the spent coffee samples compared with their roasted counterparts ($p < 0.001$). The reduction of total tannins amounted to 51–66% (Fig. 1), indicating that coffee tannins are highly soluble in hot water during espresso extraction. The total phenolic content of spent coffee ranged from 19.3 to 25.5 mg GAE/g (Fig. 2). This is similar to the result of Panusa et al. [12], who demonstrated that total phenolic content of spent coffee was between 17.1 and 35.5 mg GAE/g. However, this is slightly higher than the value found by Mussato et al. [2], who found 16 mg GAE/g of total phenolic content in the methanolic extract in spent coffee.

Antioxidative compounds

Five peaks were separated from the methanolic extracts of roasted and spent coffee (Fig. 3). The elution order was in

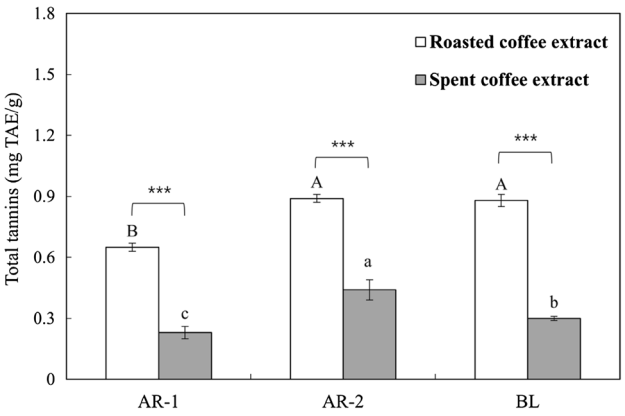


Fig. 1 Changes in total tannin content in the methanolic extracts of roasted and spent coffee. *Bars* labeled with *different letters* indicate significant differences within roasted and spent coffee, respectively, at $p < 0.05$. *Error bars* represent standard deviations from triplicate determinations. *** Indicates a significant difference at $p < 0.001$

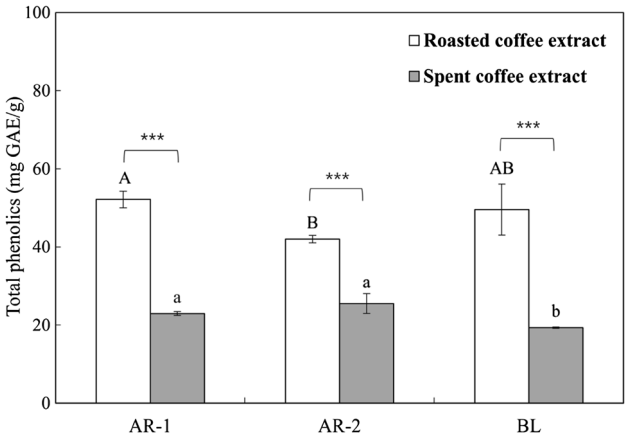
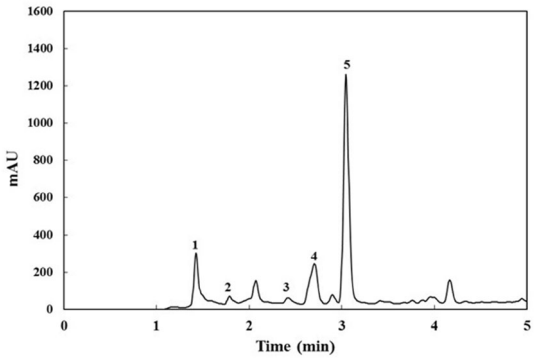


Fig. 2 Changes in total phenolic contents in the methanolic extracts of roasted and spent coffee. *Bars* labeled with *different letters* indicate significant differences within roasted and spent coffee, respectively, at $p < 0.05$. *Error bars* represent standard deviations from triplicate determinations. *** Indicates a significant difference at $p < 0.001$



Peak	t_R (min)	λ_{max} (nm)	$[M + H]^+$ (*m/z*)	Compound
1	1.4	268	138	Trigonelline
2	1.8	271	171	Gallic acid
3	2.4	272	155	Protocatechuic acid
4	2.6	325	355	Chlorogenic acid
5	3.0	274	195	Caffeine

Fig. 3 HPLC chromatogram and MS data of the coffee extract

accordance with the five antioxidant compounds identified by Yen et al. [19]. Each compound was identified by comparing the retention time and UV–visible spectrum with an authentic standard. In addition, these compounds were confirmed by the mass spectra obtained from the UPLC-qTOF-MS. These compounds were assigned as phenolic compounds, namely gallic acid (*m/z* 171), protocatechuic acid (*m/z* 155), and chlorogenic acid (*m/z* 355), and nitrogenous compounds including trigonelline (*m/z* 138) and caffeine (*m/z* 195).

Table 1 Contents of phenolic and nitrogenous compounds in the methanolic extracts of roasted and spent coffee

Peak	Compounds	AR-1			AR-2			BL		
		Roasted coffee (mg/mL)	Spent coffee (mg/mL)	Retention (%)	Roasted coffee (mg/mL)	Spent coffee (mg/mL)	Retention (%)	Roasted coffee (mg/mL)	Spent coffee (mg/mL)	Retention (%)
1	Trigonelline	7.2 ± 0.1^{b}	2.4 ± 0.1^{c}	33.3	1.9 ± 0.1^{b}	0.4 ± 0.0^{cd}	22.4	4.8 ± 0.2^{c}	0.4 ± 0.0^{c}	9.1
2	Gallic acid	2.3 ± 0.4^{c}	0.3 ± 0.2^{d}	13.3	2.5 ± 0.2^{b}	0.5 ± 0.1^{bc}	4.6	3.0 ± 0.2^{d}	0.1 ± 0.1^{d}	3.7
3	Protocatechuic acid	0.7 ± 0.0^{d}	0.2 ± 0.0^{d}	24.3	0.6 ± 0.2^{b}	0.1 ± 0.0^{d}	4.4	0.8 ± 0.0^{e}	0.1 ± 0.0^{d}	10.7
4	Chlorogenic acid	11.7 ± 0.1^{a}	3.8 ± 0.1^{b}	32.7	2.7 ± 0.4^{b}	0.8 ± 0.1^{b}	30.5	7.2 ± 0.3^{b}	1.2 ± 0.0^{b}	16.3
5	Caffeine	11.7 ± 0.1^{a}	4.2 ± 0.1^{a}	35.9	14.4 ± 4.6^{a}	4.9 ± 0.4^{a}	34.0	10.8 ± 0.5^{a}	2.5 ± 0.1^{a}	23.1

Retention (%): spent coffee (mg/mL)/roasted coffee (mg/mL) × 100. Different letters in the same column indicate a significant difference at $\alpha = 0.05$

AR arabica coffee, *BL* blended coffee

The contents of the phenolic and nitrogenous compounds in the methanolic extracts of roasted and spent coffee are presented in Table 1. Of the compounds identified in this study, chlorogenic acid and caffeine were the most abundant in both roasted and spent coffee. The retention of chlorogenic acid in spent coffee was 16–33%. This is within the range of Lopez-Barrera et al. [20], who demonstrated that 23–68% of chlorogenic acid remained in spent coffee. Similarly, 23–36% of caffeine remained in the spent coffee. In comparison, smaller percentages of gallic and protocatechuic acid were retained, which corresponded to 4–13 and 4–24%, respectively. These findings show that the retention rates of each compound varied because of the solubility in water and the stability at high temperature.

Antioxidant activity

The DPPH radical scavenging activity of the methanolic extracts of roasted and spent coffee ranged from 86.3 to 92.2% (Table 2). It is remarkable that more than 95% of DPPH activity was retained in the spent coffee. The coffee extracts show similar scavenging activity in comparison with ascorbic acid and tocopherol. Despite a substantial reduction of phenolic and nitrogenous compounds (Table 1), DPPH radical scavenging activities did not proportionally decrease. This indicates that the loss of antioxidative compounds was not accompanied by a decrease in the DPPH radical scavenging activity. Liang and Kitts [21] demonstrated that DPPH assay has a limitation when used to measure the antioxidant activity of brewed coffee because a color of the coffee potentially interferes with the DPPH absorption. Additionally, DPPH is a lipophilic radical with limited accessibility to the hydrophilic components present in coffee extract. This suggests that highly lipophilic antioxidants were not released into the hot water during the espresso extraction but remained in the spent coffee. Sridevi and Giridhar [22] reported the DPPH radical scavenging activity of free diterpenes, namely cafestol and kahweol, extracted from coffee. These diterpenes are soluble in organic solvents such as alcohol. These findings imply that the antioxidant activity of coffee is due to hydrophilic as well as lipophilic compounds.

The SOD-like activity of roasted coffee was between 48.8 and 52.7% (Table 2). This is much lower compared with the DPPH radical scavenging activity. Retention of the SOD-like activity in spent coffee ranged from 64.8 to 89.6%. The coffee extracts had higher activity than the positive control tocopherol and similar activity compared with ascorbic acid.

The FRAP values of the roasted coffee ranged from 151.4 to 187.6 mmol/mL (Table 2). This is higher than the result garnered by Yashin et al. [23], who reported that the FRAP value was 129.4 mmol/mL in coffee. The retention of FRAP in the spent coffee was 45.5–60.3%, which is almost half compared with the retention (95%) of the DPPH radical scavenging activity. This indicates that the major components contributing to DPPH and FRAP were different. Ascorbic acid and tocopherol have much higher reducing power than coffee extracts. Maillard reaction products generated from a model system of simulated bread crusts exhibited FRAP [24]. In another study, melanoidin constituted up to 25% of the dry matter of coffee beverages [25]. These data suggest that Maillard reaction products arising from coffee roasting contribute to the FRAP activity. Further studies are needed to elucidate the contribution of Maillard reaction products to the FRAP activity of spent coffee.

Correlation between total tannin content, total phenolic content, phenolic compounds, and antioxidant activity

To compare the contribution of individual compounds to antioxidant activity, Pearson's correlation coefficient is presented in Table 3. The DPPH radical scavenging activity correlated with total phenolic content in roasted and spent coffee. Chlorogenic acid showed a positive correlation with total phenolic content, as well as FRAP, only in roasted coffee. Gallic acid had a positive correlation with SOD-like activity in roasted and spent coffee,

Table 2 DPPH activity, superoxide dismutase (SOD-like) activity, and FRAP of roasted and spent coffee extracts

	DPPH radical scavenging activity (%)			SOD-like activity (%)			FRAP ($FeSO_4$ mmol/mL)		
	Roasted coffee	Spent coffee	Retention (%)	Roasted coffee	Spent coffee	Retention (%)	Roasted coffee	Spent coffee	Retention (%)
AR-1	90.8 ± 0.1^{b}	89.6 ± 0.0^{b}	98.7	49.1 ± 1.0^{b}	39.0 ± 0.5^{b}	79.4	187.6 ± 2.7^{a}	101.0 ± 5.0^{a}	53.8
AR-2	92.2 ± 0.1^{a}	91.7 ± 0.1^{a}	99.5	48.8 ± 1.8^{b}	43.7 ± 0.5^{a}	64.8	151.4 ± 1.7^{c}	91.2 ± 16.8^{a}	60.3
BL	90.1 ± 0.6^{c}	86.3 ± 1.3^{c}	95.9	52.7 ± 0.8^{a}	34.2 ± 0.0^{c}	89.6	174.3 ± 5.8^{b}	79.2 ± 6.4^{a}	45.5

Retention (%): spent coffee/roasted coffee × 100. Different letters in the same column indicate a significant difference at $\alpha = 0.05$

AR arabica coffee, *BL* blended coffee

Table 3 Pearson's correlation coefficients between total tannins content, total phenolic content, phenolic compounds, nitrogenous compounds, and antioxidant activities in the methanolic extracts of roasted and spent coffee

	Total phenolics	Total tannins	DPPH	SOD-like[a]	FRAP[b]
Roasted coffee					
Trigonelline	0.809**	–0.791*	−0.651	0.120	0.980**
Gallic acid	0.228	0.551	−0.456	0.677*	−0.068
Protocatechuic acid	0.700*	−0.317	−0.707*	0.208	0.709*
Chlorogenic acid	0.778*	−0.819**	−0.601	0.069	0.969**
Caffeine	−0.431	0.256	0.523	−0.100	−0.482
Total phenolic		−0.391	−0.791*	0.325	0.875**
Total tannin			0.089	0.431	−0.701*
DPPH				−0.720*	−0.738*
SOD-like					0.177
Spent coffee					
Trigonelline	0.118	−0.742*	0.123	−0.006	0.604
Gallic acid	0.828**	0.480	0.832**	0.851**	0.360
Protocatechuic acid	0.407	−0.512	0.410	0.300	0.716*
Chlorogenic acid	0.031	−0.793**	0.025	−0.106	0.566
Caffeine	0.951**	0.400	0.937**	0.944**	0.600
Total phenolic		0.482	0.857**	0.880**	0.539
Total tannin			0.478	0.613	−0.308
DPPH				0.953**	0.440
SOD-like					0.357

[a] Superoxide dismutase (SOD)-like activity

[b] Ferric reducing antioxidant power

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

whereas protocatechuic acid showed a positive correlation with FRAP in roasted and spent coffee. Trigonelline had a positive correlation with FRAP in roasted coffee. These findings indicate that major compounds contributing to each radical scavenging activity and reducing power are different. Further studies are needed to elucidate the contribution of each compound to the antioxidant activity of spent coffee.

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Compliance with ethical standards

Conflict of interest The authors declare no conflict of interest.

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