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Antioxidant Properties of Roasted Coffee Residues

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The antioxidant activity of roasted coffee residues was evaluated. Extraction with four solvents (water, methanol, ethanol, and *n*-hexane) showed that water extracts of roasted coffee residues (WERCR) produced higher yields and gave better protection for lipid peroxidation. WERCR showed a remarkable protective effect on oxidative damage of protein. In addition, WERCR showed scavenging of free radicals as well as the reducing ability and to bind ferrous ions, indicating that WERCR acts as both primary and secondary antioxidants. The HPLC analyses showed that phenolic acids (chlorogenic acid and caffeic acid) and nonphenolic compounds [caffeine, trigonelline, nicotinic acid, and 5-(hydroxymethyl)furfuraldehyde] remained in roasted coffee residues. These compounds showed a protective effect on a liposome model system. The concentrations of flavonoids and polyphenolic compounds in roasted coffee residues were 8,400 and 20,400 ppm, respectively. In addition, the Maillard reaction products (MRPs) remaining in roasted coffee residues were believed to show antioxidant activity. These data indicate that roasted coffee residues have excellent potential for use as a natural antioxidant source because the antioxidant compounds remained in roasted coffee residues.

KEYWORDS: Antioxidant activity; coffee residue; lipid peroxidation; free radical; chlorogenic acid; caffeic acid

INTRODUCTION

Coffee is one of the most popular and widely consumed beverages through the world due to its pleasant taste and aroma. To examine coffee, caffeine has usually attracted most attention. Except caffeine, the bioactivity and the pharmacological effects as a result of naturally occurring phenolic compounds and Maillard reaction products (MRPs) developed during the roasting process (*1*) are still unclear. Experimental evidence has shown that coffee has high antioxidant activities (*2*). In contrast, some literature has reported that roasted coffee has been found to possess mutagenic activity (*3*) and prooxidant activity (*4*). However, the same research group also noted that roasted coffee could act as a potent antioxidant and inhibit lipid peroxidation in a model system (*5*). This implies that a compound or a system which acts as antioxidant will not necessarily act in the same manner in a different model system.

Coffee grounds are the residues of the soluble coffee processing. With improvements of soluble coffee preparing processing, the daily volume of coffee residues is initially produced from 1.86 kg dropped to 0.91 kg for each kilogram of soluble coffee (*6*). The volume of coffee residues is decreased, however, their disposal still become a matter of great concern. Hence, more rational uses of roasted coffee residues have become necessary. Many attempts have been made to use roasted coffee residues as a fertilizer, animal feed, or substitutes for industrial materials; however, the best confirmed use of coffee residues is as a fuel (*6*). Although the antioxidant activity of roasted coffee in model systems is widely documented (*7*), no reports have been reported on the antioxidant activity of roasted coffee residues so far. The antioxidant properties present roasted coffee residues are expected to have interesting implications concerning lipid stability and health consequences. In addition, substitution of synthetic antioxidants whose safety is concerned by natural ones might be beneficial due to their health implication and functionality in food system. Thus, this investigation studies the antioxidant activity of roasted coffee residues.

EXPERIMENTAL PROCEDURES

Materials. Medium-roasted samples of *Coffea arabica* coffee beans were kindly donated by the Yeuan-Yuou Enterprise Co, Ltd, Taiwan, Republic of China. Roasted and ground coffee (50 g) were extracted with 95 °C water (400 mL) for 30 min, and the final ground coffee residues were dried at 50 °C for 48 h. 1,1-Diphenyl-2-picrylhydrazyl (DPPH) was purchased from Fluka Chemie AG (Buchs, Switzerland). All other chemical reagents were analytical grade and purchased from Sigma Chemical Co. (St. Louis, MO).

Sample Preparation. The roasted coffee residues of 50 g were extracted overnight with 500 mL of methanol, ethanol, and *n*-hexane, respectively, in a shaking incubator at 25 °C. The extracts were filtered and the extraction was repeated. The combined filtrates were evaporated to dryness in vacuo and weighed to determine the yield of soluble

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constituents. For water extraction, roasted coffee residues of 50 g were extracted with 500 mL boiling water for 5 min, after which the filtrate was freeze-dried and weighted. The sample was named as water extracts of roasted coffee residues (WERCR).

Antioxidant Action of Roasted Coffee Residue on Liposome Oxidation. Lecithin (580 mg) was sonicated in an ultrasonic cleaner (Branson 8210, Branson Ultrasonic Corporation, Danbury, CT) in 58 mL, 10 mmol/L phosphate buffer (pH 7.4) for 2 h in ice-cold water bath. The sonicated solution (10 mg of lecithin/mL), $FeCl_3$, ascorbic acid, and extracts (0.2 mL, 0.25−2.5 mg/mL) were mixed to produce a final concentration of 3.12 μM $FeCl_3$ and 125 μM ascorbic acid. The mixture was incubated for 1 h at 37 °C by the thiobarbituric acid (TBA) method (*8*). The absorbance of the sample was read at 532 nm against a blank, which contained all reagents except lecithin, and using a Hitachi UV-3210 spectrophotometer (Hitachi, Tokyo, Japan). These tests were run in duplicate, and analyses of all samples were run in triplicate and averaged.

Scavenging Effect of Roasted Coffee Residue on DPPH Radical. The effect of extracts on DPPH radical was estimated according to the method of Hatano et al. (*9*). The WERCR (0.2 mL, 0.25−2.5 mg/mL) were added to a methanolic solution (0.5 mL) of DPPH radical (final concentration of DPPH was 0.2 mmol/L). The mixture was shaken vigorously and allowed to stand at room temperature for 30 min; the absorbance of the resulting solution was then measured spectrophotometrically at 517 nm.

Protective Effect of Roasted Coffee Residue on Protein Oxidation. The effects of WERCR on protein oxidation were carried out according to the method of Lenz et al. (*10*). The reaction mixture (1.2 mL), containing sample (0.25−2.5 mg/mL), phosphate buffer (20 mM, pH 7.4), bovine serum albumin (20 mg/mL), $FeCl_3$ (100 μM), H_2O_2 (2.0 mM), ascorbic acid (200 μM) was incubated for 1 h at 37 °C, and 1 mL 20 mM DNPH in 2 M HCl was added to the reaction mixture. A total of 1 mL of cold TCA (20%, w/v) was added to the mixture and centrifuged at 3000*g* for 10min. The protein was washed three times with 2 mL of ethanol/ethyl acetate (1:1, v/v) and dissolved in 2 mL of 6 M guanidine-HCl (pH 6.5). The absorbance of the sample was read at 370 mM.

Determination of Reducing Activity in Roasted Coffee Residues. The reducing power of WERCR was determined according to the method of Oyaizu (*11*). WERCR (0−500 mg/mL) in phosphate buffer (2.5 mL, 0.2 M, pH 6.6) were added to potassium ferricyanide (2.5 mL, 10 mg/mL), and the mixture was incubated at 50 °C for 20 min. TCA (2.5 mL, 100 mg/mL) was added to the mixture, which was then centrifuged at 650*g* for 10 min. The supernatant (2.5 mL) was mixed with distilled water (2.5 mL) and ferric chloride (0.5 mL, 1.0 mg/mL), and then the absorbance was read spectrophotometrically at 700 nm. Higher absorbance of the reaction mixture indicated greater reducing power.

Chelating Activity of Roasted Coffee Residues on Metal Ions. The chelating activity of water extracts of roasted coffee residues on Fe^{2+} was measured according to the method of Carter (*12*). Briefly, extracts (0.2 mL, 0.25−2.5 mg/mL) were incubated with 0.05 mL of $FeCl_2 \cdot 4H_2O$ (2.0 mM). The reaction mixture was initiated by the addition of 0.2 mL of ferrozine (5.0 mM), and finally quantified to 0.8 mL with methanol. After the mixture had reached equilibrium (10 min), the absorbance at 562 nm was read. EDTA served as the positive control, and a sample without extracts and EDTA served as the negative control.

Determination of Total Polyphenolic Compounds in Roasted Coffee Residues. The concentration of phenolic compounds was measured according to the method of Taga et al. (*13*) and calculated using gallic acid as the standard. A sample (0.1 mL) was added to 2.0 mL of 2.0% Na_2CO_3. After 2 min, 50% Folin−Ciocalteu reagent (100 μL) was added to the mixture, which was then left for 30 min. Absorbance was measured at 750 nm.

Determination of Flavonoid Content in Roasted Coffee Residues. The flavonoid content of roasted coffee and roasted coffee residues was measured according to the method of Hairi et al. (*14*). Briefly, the sample was filtered and diluted with distilled water, and absorption was measured at 404 nm after the addition of 100 μL of 1% (2-aminoethyl) diphenylborate solution. The sample absorption was compared to that of a standard rutin curve.

Determination of Browning Index in Roasted Coffee Residues. The water extracts of roasted coffee and coffee residue were filtered and diluted with distilled water. The absorbance of sample solution was read at 420 mm. A high absorbance value indicated high browning intensity.

High-Performance Liquid Chromatography (HPLC) Analysis of Roasted Coffee Residues. HPLC analyses of phenolic and nonphenolic compounds were based on the method of Hakkinen et al. (*15*). HPLC was performed with a Hitachi liquid chromatograph (Hitachi, Ltd., Tokyo, Japan), consisting of a model L-7100 pump, and a model L-7455 photodiode array detector (280 nm). WERCR (50 mg/mL) was filtered through a 0.45 μm filter and injected onto the HPLC column. The injection volume was 20 μL, and the flow rate was 1.0 mL/min. The separation temperature was 25 °C. The column (5 μm, 250 × 4 mm i.d.) was a Lichrosorb RP 18 (5 μm, 10 mm × 4 mm). The solvents were as follows: (A) 50 mM ammonium dihydrogen phosphate, pH 2.6; (B) 0.2 mM *o*-phosphoric acid, pH 1.5; and (C) 20% solvent A in 80% acetonitrile. The solvent gradient elution program was as follows:

time (min)	solvent (%)		
	A	B	C
0	100	0	0
5	96	0	4
10	92	0	8
10.1	0	92	8
20	0	80	20
30	0	70	30
60	0	50	50
75	0	20	80
80	100	0	0

RESULTS AND DISCUSSION

The yields of roasted coffee residues extracted with various solvents in the order of the yield as *n*-hexane > water > ethanol > methanol. Of the four solvent extracts, the yield of hexane extracts (8.4%) and water extracts (6.58%) was about 6- and 4.7-fold greater than that of ethanol extracts (1.38%), respectively. These results show that hexane and water are effective in extracting roasted coffee residues and give higher yields of extracts than the other solvents. Gross et al. (*16*) reported that coffee beans contained 8.7−12.2 g/kg lipid material, and the lipid classes in coffee been lipids were identified as triacyl-

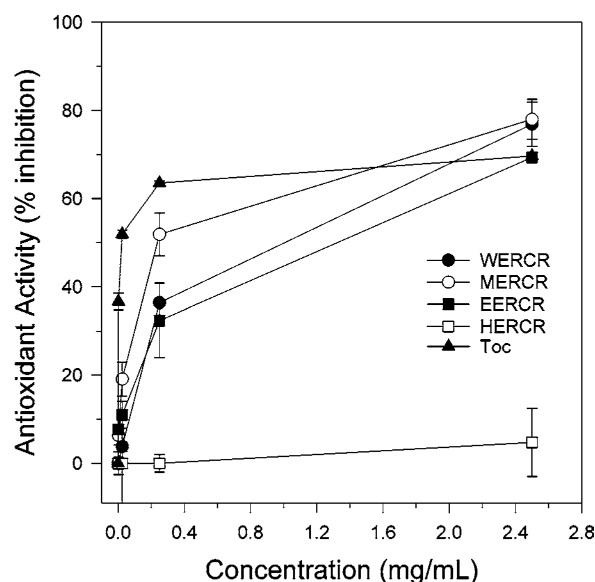

Figure 1. Inhibitory effect of roasted coffee residues with different solvents in liposome model system.

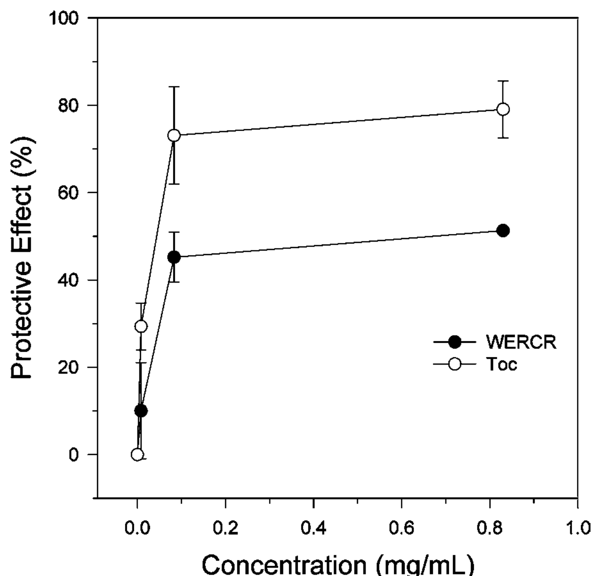

Figure 2. Protective effect of water extracts of roasted coffee residues (WERCR) on protein oxidative damage. Toc, tocopherol.

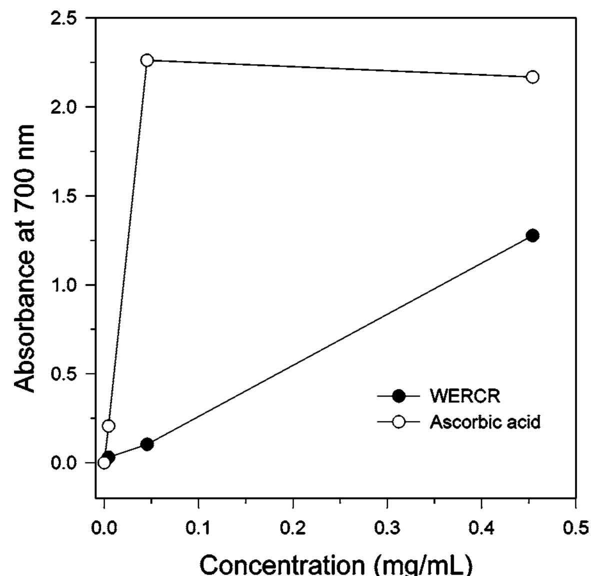

Figure 4. Reducing ability of water extracts of roasted coffee residues (WERCR). AA, ascorbic acid.

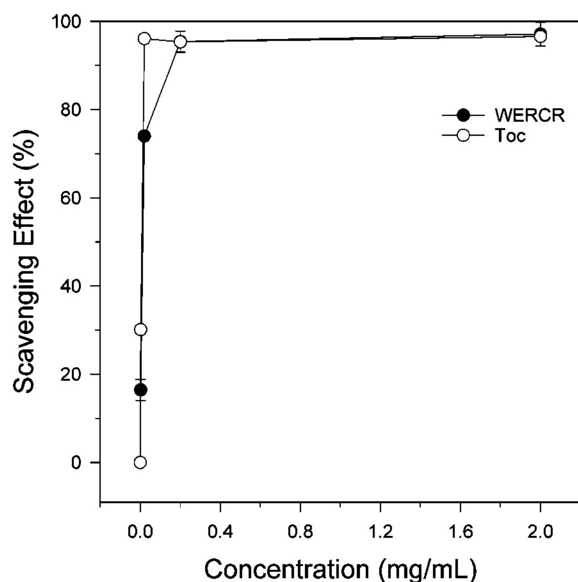

Figure 3. Scavenging effect of water extracts of roasted coffee residues (WERCR) on free radical. Toc, tocopherol.

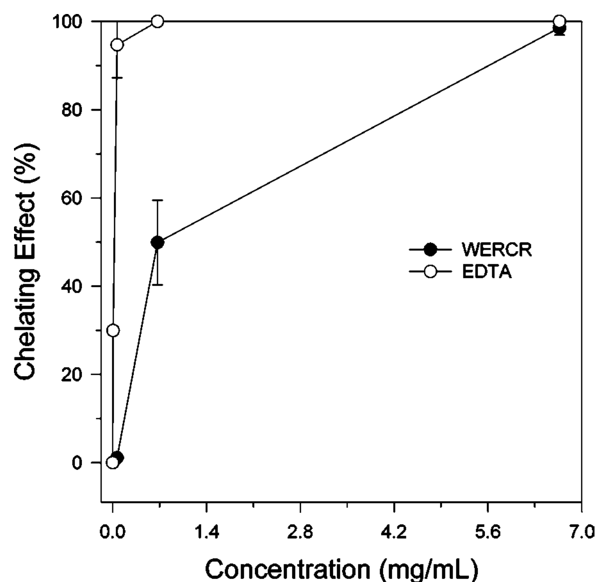

Figure 5. Chelating effect of water extracts of roasted coffee residues (WERCR) on iron ions. EDTA, ethylenediamine tetraacetic acid.

glycerols, terpene esters, free sterols, sterol esters, free fatty acids, partial acylglycerols, and polar lipids, which may explain why hexane was able to produce the highest yield of extracts.

Figure 1 shows the antioxidant activity of extracts from roasted coffee residues with various solvents in liposome model system, as compared with tocopherol (Toc). Of the four solvent extracts, water extracts, methaol extracts, and ethanol extracts of roasted coffee residues displayed antioxidant activity, and the antioxidant activity of three samples increased with increasing the concentration of the extracts, indicating that water extracts of roasted coffee residues (WERCR), methanol extracts of roasted coffee residues (MERCR), and ethanol extracts of roasted coffee residues (EERCR) produced the most significantly inhibitory effect on liposome peroxidation. However, hexane extracts of roasted coffee residues (HERCR) showed almost no inhibitory effect on liposome peroxidation, revealing that lipophilic components were not a major component in roasted coffee residues as antioxidant. According to the data in **Figure l**, WERCR tended to be better protection for lipid peroxidation and gave higher yields. Thus, WERCR was the focus of the following study.

Protective Effect of WERCR on Protein Oxidation. Reactive oxygen species may react with amino acids and can alter protein structure and function (*17*). In addition, some amino acid residues are oxidized to carbonyl derivatives, so the carbonyl content of proteins can be used as a measure of protein oxidation (*18*). The protective action of WERCR on protein carbonyl formation in albumin in a reaction mixture containing $FeCl_3$, H_2O_2, and ascorbic acid is shown in **Figure 2**. From the data reported in **Figure 2**, it can be observed that in the range of 0−0.8 mg/mL of WERCR and Toc used, the protective action against protein oxidation increased with increasing concentration of samples.

The protective action of WERCR on protein oxidation was somewhat less effective than Toc. Ames et al. (*19*) noted that lipofuscin, a peroxidized lipid−protein aggregate, accumulates during aging or under conditions of oxidative stress, which may occur as a result of declines in antioxidant defenses or loss of

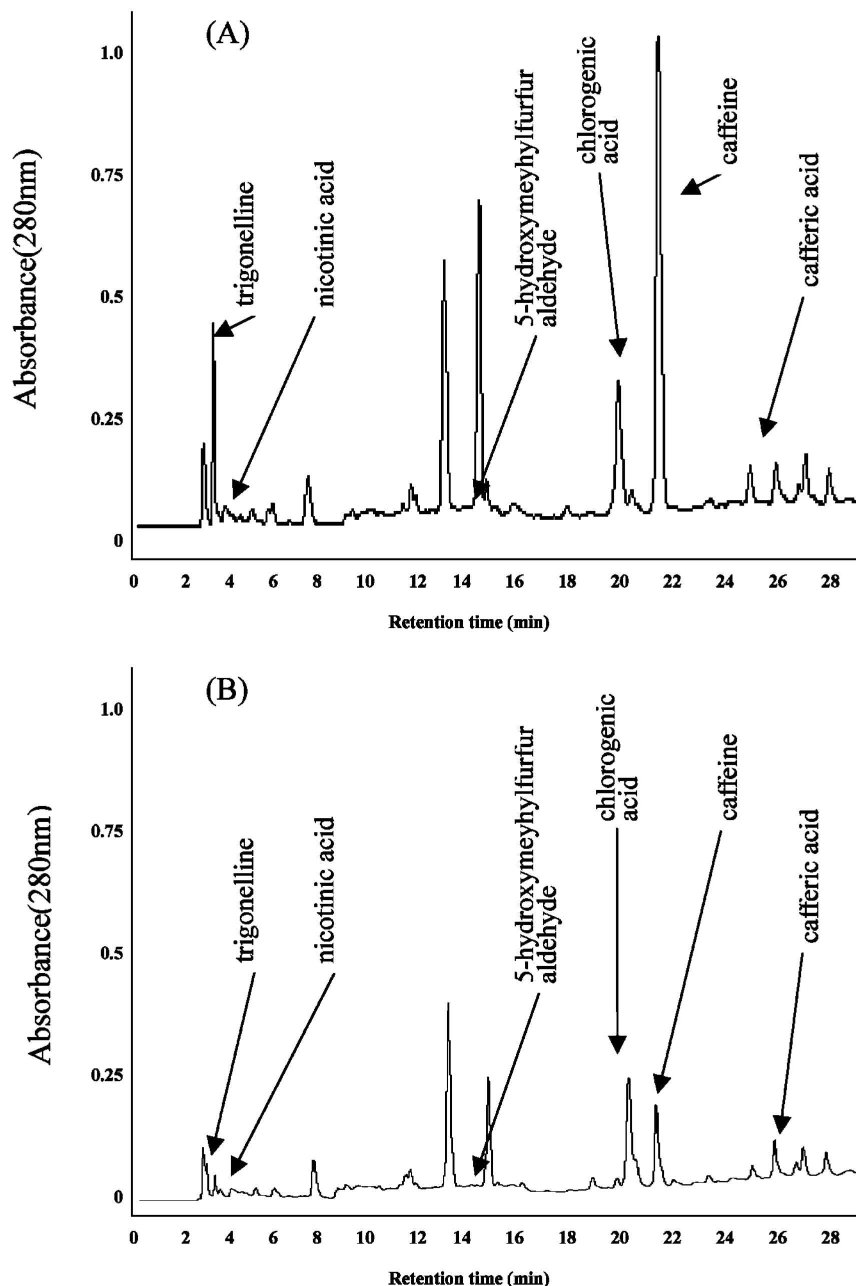

Figure 6. HPLC chromatogram of roasted coffee (**A**) and roasted coffee residue (**B**).

repair functions. In other words, with more antioxidants in the diet, there is more protective effect against oxidative stress. In the present work, WERCR is shown to have an inhibitory effect on oxidative damage and to stabilize protein against oxidation. This finding implies that roasted coffee residues may be used as resources in attempts to protect from oxidative stress in human disease.

Scavenging Free Radical by WERCR. The free radical scavenging activity of WERCR was determined using 2,2-diphinyl-1-picrylhydrazyl radical (DPPH•). The model systems allowed WERCR to react with DPPH• in a chloroform solution. The reduction of DPPH•, indicating a positive antiradical activity of WERCR, was followed by monitoring the decrease in its absorbance at 517 nm during the reaction (*20*). In other words, the change in absorbance produced by reduced DPPH• was used to evaluate the ability of WERCR to act as free radical scavengers. As can be seen in **Figure 3**, DPPH decolorization was increased by WERCR in a concentration-dependent manner. WERCR and Toc at 0.2 mg/mL showed 95.4 and 95.3% scavenging effect on the DPPH radical, respectively, and no significant difference ($p > 0.05$) in scavenging effect on the DPPH radical was found between Toc and WERCR. According to the DPPH tests, WERCR acted as a direct free radical scavenger, and its potency was similar to that of Toc, indicating that WERCR had the greatest antiradical activity.

Reducing Ability of WERCR. Antioxidant activity of antioxidants has been shown by some researchers to be concomitant with the development of reducing power (*21*). In **Figure 4**, the reducing power increased with increasing concentration of WERCR. Although the reducing power of WERCR is less than ascorbic acid, WERCR exhibited potent reducing ability, which may contribute to the antioxidant activity of WERCR. The results suggested that the reducing agents in WERCR may be derived from some naturally occurring materials remained in residues.

Binding Activity of WERCR on Ferrous Ions. Figure 5 shows that the metal-binding activity by WERCR on ferrous ions increased with increasing concentrations of WERCR.

Table 1. Concentration of Phenolic and Nonphenolic Compounds in Water Extracts of Roasted Coffee and Roasted Coffee Residues

compound	concentration (mg/mL)	
	roasted coffee	roasted coffee residues
chlorogenic acid	5.780 ± 0.47	0.490 ± 0.063
caffeic acid	0.150 ± 0.014	0.148 ± 0.026
caffeine	8.370 ± 0.28	0.640 ± 0.076
trigonelline	15.240 ± 2.15	11.250 ± 1.32
nicotinic acid	1.660 ± 0.36	0.170 ± 0.04
5-(hydroxymethyl)-furfuraldehyde	0.040 ± 0.004	0.008 ± 0.0004

WERCR at 0.67 mg/mL showed 49.9% binding activity, whereas 6.67 mg/mL showed a complete binding effect (98.6%) on ferrous ions. Iron as a transition metal is of interest as a catalyst of lipid peroxidation due to its biological relevance and the ability to react with oxygen to form species capable of initiating peroxidation or of reacting directly with lipid peroxides, propagating the reaction (*22*). In the present study, WERCR displayed a remarkable capacity for iron binding, suggesting that its action as peroxidation protector may be in part related to iron interaction. Although the binding effect of WERCR is relatively small when compared with that of EDTA, it is significant because WERCR exerted to combine with high affinity with ferrous ions that minimize the concentration of the catalyzing transition metal in lipid peroxidative reaction.

On the basis of the data obtained from **Figures 3−5**, the protective effects of WERCR on oxidative damage of liposomes and protein may be the consequence of its radical scavenging, reducing ability, and iron-binding capacity, or both or all of these activities.

Analysis of Antioxidant Compounds in WERCR. Some studies have shown that bioactive compounds such as chlorogenic acid, caffein, caffcic acid, nicotinic acid, trigonelline, and 5-(hydroxymethyl)furfuralolehyde were found in the coffee (*8*, *23*, *24*). Thus, it is necessary to explore the antioxidant compounds in roasted coffee residues. In the present study, the bioactive compounds (phenolic and nonphenolic compounds) mentioned above were determined. The analytical plot is shown in **Figure 6**. **Table 1** shows the concentration of phenolic and nonphenolic compounds in roasted coffee and roasted coffee residues. The concentration of phenolic and nonphenolic compounds in roasted coffee residues are less than in roasted coffee, which is suggested because most soluble compounds have been extracted in roasted coffee brews, reducing the remainder of soluble compounds in roasted coffee residues. **Figure 7** shows that the inhibitory effect of phenolic and nonphenolic compounds in roasted coffee residues in a liposome model system, as compared with WERCR and Toc. The different concentrations of samples were prepared and their antioxidant activity was determined. In general, the antioxidant activity of WERCR is significantly higher than other samples, which may be due to the synergism of the components with one another. Of the samples tested, chlorogenic acid and 5-(hydroxymethyl)furfuraldehyde exhibited marked inhibitory effect on liposome oxidation; however, caffeine, nicotinic acid, and trigonelline showed lower antioxidant activity. This implies that nonphenolic compounds including caffeine, nicotinic acid, and trigonelline displayed less inhibitory effect on liposome oxidation. Chlorogenic acid, caffeic acid, and related compounds are well-known to be antioxidants (*25*). Laranjinha et al. (*26*) noted that naturally occurring phenolic acids (chlorogenic acid and caffeic acid) prevent ferrylmyoglobin-dependent LDL oxidation. Salvi et al. (*27*) noted that chlorogenic acid protected

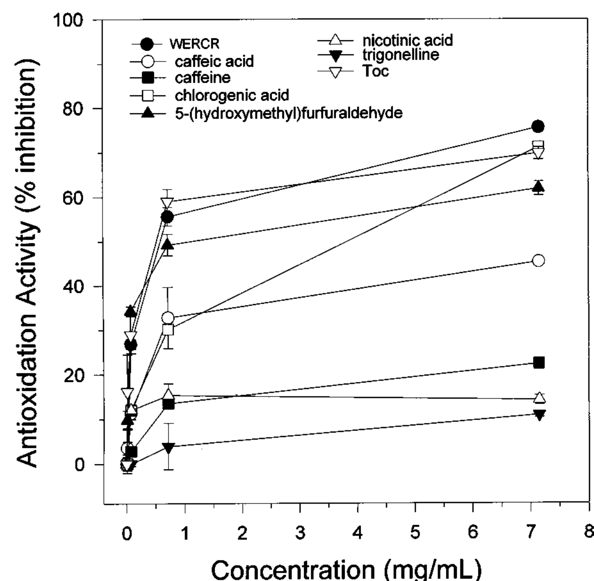

Figure 7. Inhibitory effect of phenolic and nonphenolic compounds in water extracts of roasted coffee residues (WERCR) in liposome model system. Toc, tocopherol.

oxidative damage of proteins as a result of scavenging and protective activities correlated well with the amount of free radicals generated. Caffeine was an effective inhibitor of oxidative damage induced by reactive oxygen species in membranes (*28*). Although no reports of nicotinic acid, trigonelline, and 5-(hydroxymethyl)furfuraldehyde against lipid peroxidation have been reported so far, three samples in this work showed an antioxidant effect on liposome peroxidation. In addition, the concentration of flavonoids and polyphenols in WERCR was 8400 and 20 400 ppm, respectively, which was less than in roasted coffee (14 400 and 21 600 ppm, respectively). These compounds, which can be chemically lost and/or transformed during the heating process, may also contribute to the antioxidant effects of roasted coffee residues.

Anese and Nicoli (*29*) reported that ready-to-drink coffee brews have high antioxidant properties partially due to their reducing properties, which are mainly attributed to the Maillard reaction products (MRPs), formed during the heating process, and these brown polymers are known to have strong reducing power. Furthermore, MRPs were found to act also as oxygen scavengers (*30*). In this study, the browning index in WERCR and roasted coffee brews was 0.271 and 0.305, respectively, indicating that WERCR had lost some portion of their MRPs. However, the MRPs remaining in WERCR may contribute to its antioxidant activity (*31*). As for the iron, polyphenolics such as chlorogenic acid formed an inactive Fe^{2+}−polyphenolics complex by reducing Fe^{3+}, consequently, diminished the Fenton reaction (*32*). It can be assumed that the protective effect on lipid and protein oxidation and chelating action on iron as well as reduce hydroperoxide to nonradical products by WERCR might in part be the result of nonphenolic and polyphenolic compounds as well as MRPs.

In conclusion, the results reported in this study indicate that water extracts of roasted coffee residues show strong overall antioxidant properties, which may mainly be attributed to the polyphenolic and nonpolyphenolic compounds. These compounds can act both as primary and secondary antioxidants. MRPs formed during the heating process remained in roasted coffee residues and may also contribute to the antioxidant effects of roasted coffee residues. In addition, considering the large consumption of coffee in the world, these results should be

useful because they indicate that roasted coffee residues can be used as a potential natural antioxidant.

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