Antioxidant Effect of Extracts from the Coffee Residue in Raw and Cooked Meat

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Summary and Implications

This study evaluated the antioxidant activity of ethanol or hot water extracts from the residues of coffee after brewing. The extraction experiment was carried out using conventional solid–liquid methods, including ethanol and water as the extraction media at different temperatures and liquid/solid ratios. The antioxidant activity of extracts was tested for total phenolic compounds (TPC), 2, 2-diphenyl-1-picrylhydrazyl (DPPH), and 2-thiobariburic acid reactive substances (TBARS) using oil emulsion and raw/cooked meat systems. The DPPH radical scavenging activity of the ethanol extracts with heating (HEE) and without heating (CEE) were higher than that of the hot water extracts (WE). The highest DPPH value of HEE and CEE at 1000 ppm was 91.22% and 90.21%, respectively. In oil emulsion and raw/cooked meat systems, both of water and ethanol extracts had similar antioxidant effects to the positive control (BHA), but HEE and CEE extracts showed stronger antioxidant activities than WE extract. The ethanol extracts of coffee residue had a strong antioxidant activity, and thus have potential to be used as a natural antioxidant in meat.

Introduction

Natural antioxidants from plant origins are safe and can replace the synthetic ones. Coffee is well known as a rich source of antioxidants that can reduce the oxidative stress in humans. Recently, the consumption of coffee around the world has increased significantly due to its positive health effects. Thousands of tons of residues after brewing the ground coffee at restaurants, cafeterias, and consumers levels are produced annually in the U.S., but all of the grounds are disposed. However, significant amounts of antioxidants can be remaining in the residues. So, if they were properly recovered, there could be an opportunity to use them as natural antioxidants. Studies showed that the extracts from the residue of brewed coffee exhibited anti-inflammatory, anti-tumor and anti-allergic activities due to the presence of phenolic compounds such as chlorogenic acid, caffeine, caffeic acid, trigonelline and protocatechuic acid. Chlorogenic acid, which is one of the most abundant phenolic compounds in the extract of coffee residue (ECR), and has been reported to have many beneficial functions, including hepatoprotective, hypoglycemic, anti-bacterial, antiviral, anti-inflammatory and anti-carcinogenic activities in humans. Various extraction techniques have been applied to recover antioxidant compounds from natural and organic sources. Solvents such as methanol, ethanol, acetone, ethyl acetate, and their combinations have been used to extract phenolics from coffee or the coffee residues, often with different proportions of water. Among these extraction methods, hot water and ethanol treatments were safe and the most commonly used extraction techniques. In other studies, roasted coffee residue was extracted with methanol, ethanol, and *n*-hexane, respectively, in a shaking incubator at 25 ^{o}C. The water extracts of roasted coffee residues showed the best antioxidant properties which might be mainly attributed to the polyphenolic and nonpolyphenolic compounds in the extract. Thus, phenol-rich extracts could be obtained from the ground coffee using an environmentally friendly and simple extraction procedure. The objective of this study was to investigate the antioxidant potential of the extract from coffee residues, and 2) to evaluate the antioxidant effect of the extract from coffee residues in an oil emulsion and raw/cooked meat system.

Material and Methods

The residues after brewing ground coffee were obtained from a local cafeteria and used as the raw material to extract antioxidant compounds. Extraction of coffee residues was performed using ethanol or water. Both water and ethanol extracts were lyophilized in a freeze dryer and stored until use. The total phenolic content in ECR was determined using the Folin-Ciocalteu's reagent. 2,2-Diphenyil-1- picrylhydrazyl (DPPH) radical scavenging capacity was determined as antioxidant capacity of the coffee extract. Lipid oxidation of raw and cooked meat was measured using the TBARS method.

Statistical Analysis

All results are presented as mean ± standard deviation (SD) and standard error of the means (SEM). Statistical analysis was performed using the SPSS for Windows. All experiments were replicated three times (n=3). Mean values were compared using the one-way

analysis of variance (ANOVA) followed by Duncan's multiple range test ($P < 0.05$).

Results and Discussion

The total phenolic compound values of ECR sample were 41.97, 35.51, and 28.10 mg/mL for HEE, CEE and WE, respectively. When the coffee residue was extracted using ethanol with heating (HEE), the amount of phenolic compounds was higher than the other two methods (ethanol extraction at room temperature and hot-water extraction) (Table 1). Some differences in phenolic compounds could be attributed to the coffee extraction process, roasting and variety of coffee products utilized. This result indicated that the residue of brewed coffee still contained significant amounts of phenolic compounds and that it could be used as a source for the phenolic antioxidants.

The water extract (WE) showed a high DPPH radical scavenging activity at a high concentration (1000 ppm). At lower concentrations, however, the antioxidant activity was not significantly different from control ($p < 0.05$). The ECR prepared with ethanol had higher DPPH radical scavenging activity than that with water. The DPPH radical scavenging activity of HEE and CEE were not significantly different ($p < 0.05$) at 250 to 1000 ppm levels. The DPPH value of HEE and CEE ranged from 38.16 to 90.39% and 37.10 to 89.05% at a concentration of 250 to 1000 ppm. However, WE of ECR showed relatively low radical scavenging activity of 12.03, 28.86 and 55.42% at 250, 500 and 1000 ppm levels, respectively.

These results indicated that all ECR have considerable DPPH radical scavenging capacity. The HEE and CEE both exhibited higher DPPH radical scavenging activity than the WE, and the ethanol extract with heating displayed the highest level of free radical scavenging activity. The stability of oil emulsion was one of the most important parts of this experiment because the oil emulsion system can show the antioxidant effects of the ECR the best. During the incubation time, there was no visible or physical change in oil emulsion samples (Table 3). At 0 hours, the TBARS values were found to be the same for all treatments and increased significantly with the increase of incubation time. The TBARS value of control increased rapidly from 0.066 to 0.365 mg MDA/L of oil emulsion. However, all ECR exhibited significantly lower TBARS values than the control. The TBARS values of WE extract increased from 0.033 to 0.331 mg MDA/L (1000 ppm) during 72 h incubation time, but it was still significantly lower than the control ($p < 0.05$). The HEE and CEE showed similar antioxidant activity to BHA (50 ppm). Higher concentrations of HEE and CEE extracts, however, did not improve the antioxidant activity significantly between 500 to 1000 ppm. After 72 h incubation, HEE and CEE at 500 to 1000 ppm showed 75.6-78.6% lower TBARS values than the control. HEE and CEE at 500 ppm was as effective as 50 ppm BHA, indicating that they can be good antioxidants for oil emulsions. These results demonstrated that HEE and CEE have a potent antioxidant activity in oil emulsions. Water had lower extraction power than ethanol because most of the water-soluble antioxidant compounds were already extracted during brewing process. The TBARS value of the control (without extracts) increased significantly ($p < 0.05$) during the first 3 hr of incubation and then remained the same (Table 4). The meat homogenate with BHA did not show any changes in TBARS during incubation. The TBARS of HEE and CEE increased significantly during the 1 hr of incubation and then remained the same or decreased after 12 hr of incubation. The TBARS of WE increased during the 3 hr of incubation and then decreased after 12 hr. The TBARS values of BHA were the lowest among the treatments, indicating that 50 ppm BHA had the stronger antioxidants than 500 and 1000 ppm of HEE, CEE or WE treatments. However, all other treatments also showed significant ($p < 0.05$) antioxidant effects during the incubation. For all the ECR (HEE), CEE and WE, 1000 ppm showed stronger antioxidant effects than 500 ppm. Considering the ethanol and water extracts, ethanol extracts showed stronger antioxidant effects at the same concentration because organic solvents are more efficient in extracting antioxidant compounds from the coffee residue. Considering the high temperature (heating) or room temperature extracts, the high temperature extract showed stronger antioxidant effects than the room-temperature extract. The TBARS values of meat homogenate with ECR were < 1.0 mg/kg, which are within the acceptable level. At the 0 day, the TBARS value of meat with the control treatment was significantly higher than that of all other treatments. The TBARS of cooked meat rapidly increased during the storage, especially in the control. The cooked meat with 140 ppm BHA nearly stopped lipid oxidation during the 5-day storage period (Table 5). ECR treatments showed varying antioxidant effects depending upon the extraction methods used: HEE extract showed the strongest and WE extract showed the weakest antioxidant effects among the ECRs. All the ECR treatments maintained low-levels of TBARS values after 1 day of storage, but the TBARS values increased significantly after 3 days of storage. The antioxidant effect of 140 ppm BHA was significantly higher than any of the ECR. The result of ECR in cooked meat system was little different from other systems (oil emulsion or raw-meat homogenate). Although, the ECR does not have strong enough antioxidant potential to

prevent lipid oxidation of cooked meat, it still showed higher antioxidant activity than the control. These results indicate that activity of these antioxidants delayed lipid oxidation in the cooked-meat patties during storage.

Conclusions

The ethanol and water extracts of coffee residue showed significant antioxidant activity and DPPH radical scavenging capacity. Among the three different extraction methods, HEE was the best method in extracting antioxidant compounds from coffee residues. HEE was effective in preventing lipid oxidation in oil emulsion and raw meat systems, but was not strong enough to prevent oxidative changes in cooked-meat packaged in oxygen permeable bags for more than 3 days. This suggested that residues of coffee after brewing have potential to be used as a source of natural antioxidants.

Table 1. The total phenolic compounds of ECR samples.

Sample	Conc. (ppm)	TPC (mg GAE activity/g ECR)
HEE[1]	1000	$41.97^{a} \pm 2.49$
CEE		$35.51^{b} \pm 2.93$
WE		$28.10^{c} \pm 0.76$

[a-c]: Means with different letters in a column are significantly different between extraction methods (p <0.05). [1]HEE: ethanol extraction with heating, CEE: ethanol extraction with room temperature, WE: hot water extraction.

Table 2. DPPH radical scavenging activity of ECR at different concentrations.

Sample	Conc. (ppm)		
	250	500	1000
HEE[1]	$38.16^{ax} \pm 1.33$	$72.15^{ay} \pm 1.37$	$90.39^{az} \pm 0.14$
CEE	$37.10^{ax} \pm 1.28$	$69.39^{ay} \pm 0.67$	$89.05^{az} \pm 0.74$
WE	$12.03^{bx} \pm 2.76$	$28.86^{bx} \pm 1.52$	$55.42^{by} \pm 0.75$

[a-b]: Means with different lowercase letters in a column are significantly different between extraction methods (p < 0.05). [x-z]: Means with different capital letters in a row are significantly different between sample concentrations (p <0.05). [1]HEE: ethanol extraction with heating, CEE: ethanol extraction with room temperature, WE: hot water extraction. [2]SEM: standard error of mean.

Table 3. Antioxidant effect of coffee residue extracts on the TBARS (mg MDA/L of oil emulsion) of an oil emulsion model system

Sample	Con. (ppm)	Incubation time (h)					
		0	6	24	30	48	72
Control[1]	*0*	0.066^{av}	0.094^{aw}	0.182^{ax}	0.292^{ay}	0.309^{ay}	0.365^{az}
BHA	50	0.031^{bv}	0.034^{cwv}	0.038^{cw}	0.047^{cx}	0.056^{cy}	0.081^{cz}
HEE	500	0.025^{bv}	0.040^{cw}	0.051^{cx}	0.052^{cx}	0.056^{cy}	0.082^{cz}
	1000	0.027^{bv}	0.039^{cw}	0.048^{cy}	0.049^{cy}	0.049^{cy}	0.078^{cz}
CEE	500	0.026^{bx}	0.040^{cx}	0.031^{cx}	0.048^{cy}	0.061^{cy}	0.089^{cz}
	1000	0.027^{bv}	0.042^{cw}	0.050^{cx}	0.050^{cx}	0.061^{cy}	0.086^{cz}
WE	500	0.034^{bu}	0.075^{bv}	0.145^{abw}	0.222^{bx}	0.284^{by}	0.326^{bz}
	1000	0.033^{bv}	0.096^{aw}	0.112^{bw}	0.214^{bx}	0.277^{by}	0.331^{bz}

[a-c]: Means with different letters in a column are significantly different between extraction methods and concentration (p < 0.05). [u-z]: Means with different letters in a row are significantly different between incubation times (p <0.05). [1]Control: without extraction sample, BHA: 50 ppm BHA solution, HEE: ethanol extraction with heating, CEE: ethanol extraction with room temperature, WE: hot water extract.

Table 4. TBARS (mg MDA/kg of raw-meat homogenates) of meat homogenates with different ECR treatments during storage at 37 °C

Sample	Conc. (ppm)	Incubation time (hours)				
		0	1	3	6	12
Control[1]		0.190^{ax}	0.363^{ay}	0.454^{az}	0.436^{az}	0.469^{az}
BHA	50	0.150^{b}	0.191^{e}	0.193^{e}	0.191^{d}	0.192^{e}
HEE	500	0.202^{ay}	0.267^{cz}	0.261^{dz}	0.267^{cz}	0.227^{cdy}
	1000	0.202^{ayx}	0.228^{dz}	0.237^{dz}	0.219^{dzy}	0.196^{dex}
CEE	500	0.202^{ay}	0.313^{bz}	0.304^{cz}	0.351^{bz}	0.298^{bz}
	1000	0.202^{ay}	0.272^{cz}	0.256^{dz}	0.258^{cz}	0.215^{dey}
WE	500	0.202^{ax}	0.309^{by}	0.377^{bz}	0.363^{bz}	0.284^{by}
	1000	0.202^{ax}	0.273^{cz}	0.291^{cz}	0.286^{cz}	0.249^{cy}
SEM		0.008	0.010	0.016	0.016	0.018

$^{a-e}$: Means with different letters in a column are significantly different between extraction methods and concentration ($p < 0.05$). $^{x-z}$: Means with different letters in a row are significantly different between incubation times ($p < 0.05$). [1]Control: without extraction sample, BHA: 50 ppm BHA solution, HEE: ethanol extraction with heating, CEE: ethanol extraction with room temperature, WE: hot water extraction.

Table 5. TBARS values (mg MDA/kg of cooked-meat patties) of cooked chicken patties with different ECR samples during storage at 4 °C

Sample	Conc. (ppm)	Storage time (days)			
		0	1	3	5
Control[1]		0.105^{ax}	0.393^{ay}	0.807^{az}	0.790^{az}
BHA	140	0.022^{cy}	0.026^{dy}	0.040^{dz}	0.039^{dz}
HEE	1000	0.025^{cw}	0.076^{cx}	0.197^{cy}	0.263^{cz}
CEE	1000	0.031^{bx}	0.121^{by}	0.380^{bz}	0.396^{bz}
WE	1000	0.029^{bw}	0.128^{bx}	0.396^{by}	0.494^{bz}
SEM		0.005	0.023	0.048	0.048

$^{a-d}$: Means with different letters in a column are significantly different between extraction methods ($p < 0.05$). $^{w-z}$: Means with different letters in a row are significantly different between incubation times ($p < 0.05$). [1]Control: without extraction sample, BHA: 140 ppm BHA solution, HEE: ethanol extraction with heating, CEE: ethanol extraction with room temperature, WE: hot water extraction. [2]SEM: standard error of mean.