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Effect of Roasting on Properties of the Zinc-Chelating Substance in Coffee Brews

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ApV is a brownish polymer with zinc-chelating activity in brewed coffee. We investigated in this study the effects of roasting on the zinc-chelating, reducing, and antioxidative activities of ApV from light-, medium-, and dark-roasted coffee. We also discuss the effect on the zinc-chelating activity of adding milk to the brewed coffee. The chelating activities of ApVs were evaluated by the tetramethyl murexide method. As the intensity of roasting increased, the yield of ApV increased, and the brown color and molecular weight of ApV respectively became darker and higher. Increasing the degree of roasting also decreased the zinc-chelating activity of ApV. The reducing activities of ApVs estimated by the indophenol method were stronger than those of ascorbic acid. Both the antioxidative activity estimated by the ABTS assay and the reducing activity of ApV increased with roasting. When milk was added to instant coffee and its ApV was prepared, the zinc-chelating activity of ApV was not changed.

KEYWORDS: Coffee; melanoidin; chelating activity; dissociation constant; reducing activity

INTRODUCTION

Coffee consumption is popular throughout world. The roasting process that is applied to green coffee beans forms the pleasant aroma, taste, and brown color of brewed coffee. The major reactions occurring during roasting are the Maillard reaction between sugars and amino groups, and the oxidative polymerization or degradation of phenolic compounds (*1*). Green coffee beans contain about 8% phenolic compounds, 7% reduced sugars, and 12% protein (*1*). In addition, coffee has one of the richest contents of chlorogenic acid and its derivatives (*2*, *3*). The brown pigments in the coffee brew are formed during roasting by reactions among phenolic compounds, sugars, and amino acids, these compounds being easily decomposed and polymerized (*1*, *4*, *5*). Consequently, the brown pigments in brewed coffee and in roasted coffee beans may have a partial structure similar to that in melanoidins and the polymers of phenolic compounds (*6*).

The effect of brewed coffee on mineral nutrition has recently been noticed. Brewed coffee has metal-chelating activity which can result in trace element deficiency in those known geographical areas where coffee is consumed in large quantities (*6*). In addition, it has been reported that an excessive intake of brewed coffee inhibits iron absorption (*7*). Zinc is an essential trace element, with about 1.5 g of zinc being found in a human adult. It is essential for DNA and RNA syntheses, and for many enzymatic reactions, while it is also important for membrane structure and function, as well as for normal immunocompetence. Brewed coffee contains browning substances such as melanoidins and polyphenolic compounds that greatly contribute to the strong antioxidative activity and to the metal-chelating activity (*8*−*12*). The fact that browning substances can strongly chelate zinc would lead to decreased availability of zinc.

Coffee brews contain a brownish zinc-chelating polymer designated ApV (*6*, *13*). ApV was prepared from the precipitate formed in the coffee extracts by adding $ZnCl_2$ and purified using ion-exchange and cellulose column chromatographies. ApV is a brown amorphous powder that is soluble in water, but not in organic solvents, and the chemical formula of ApV from instant coffee was experimentally determined to be $C_{16}H_{21}O_9N_3$. ApV is considered to be formed by the Maillard reaction, involving the polymerization of phenolics or their degradation products during roasting (*13*), and ApV of instant coffee has shown definite chelating activity against Fe, Zn, and Cu (*14*), as well as antioxidative activity (*6*).

The addition of creamer or milk to brewed coffee is a common practice. The purpose of adding these dairy or nondairy products to the coffee beverage is to develop a desirable color change, to give body to the coffee beverage, to reduce the bitter and sour tastes, and to reduce the astringency of the coffee. Most investigations (*15*, *16*) carried out on such additives have been focused on their effect on the retention of volatiles. However, there is no information about the effect of adding milk to the metal-chelating activity of brewed coffee.

Many studies have been made on the differences among light-, medium-, and dark-roasted coffee in terms of antibacterial (*17*) and antioxidative activities (*2*). We investigated in this study the effects of roasting on the zinc-chelating, reducing, and

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antioxidative activities of ApV from light-, medium-, and dark-roasted coffee, and the relationship between the chelating and reducing activities of ApV. We also discuss the effect on the zinc-chelating activity of adding milk to brewed coffee.

MATERIALS AND METHODS

Reagents. All chemicals used were of analytical grade. Cellulose mikrokristallin was purchased from Merck Japan Ltd. (Tokyo, Japan), 2,2′-azinobis(3-ethylbenzothiazoline-6-sulfonic acid; ABTS) was purchased from Wako Pure Chemical Industries (Osaka, Japan), and tetramethyl murexide ammonium salt (TMM) was obtained from Sigma Chemical Company (St. Louis, MO).

Preparation of Coffee Samples. Robusta green coffee beans (*Coffea robusta*) cultivated in Vietnam in 2003 was supplied by Tokyo Allied Coffee Roasters Co. Ltd. (Tokyo) and stored at 4 °C before their use. Light-, medium-, and dark-roasted coffee beans were respectively roasted in an FP-31 muffle furnace (Yamato, Tokyo, Japan) at 200 °C for 30, 60, and 120 min. The values for the weight loss of the light-, medium-, and dark-roasted coffee beans were 12%, 15%, and 18%, respectively. The green and roasted coffee beans were pulverized in a National MK-51M-HW coffee mill (Matsushita, Kobe, Japan). Five hundred milliliters of hot water was added to 60 g of the coffee powder, and the mixture was stirred for 3 min. The supernatant obtained by centrifugation of the mixture was lyophilized, with the obtained powder being used as the extract of coffee beans. Nescafe Goldblend instant coffee was supplied by Nestle (Kobe, Japan).

Preparation of the Zinc-Chelating Substance from Coffee. ApV was prepared according to the method of Takenaka et al. (*13*). Ten grams each of lyophilized powder of the hot water extracts obtained from green and roasted coffee beans were suspended in 30 mL of a 20 mM $ZnCl_2$/10 mM hexamethylenetetramine·HCl buffer solution (pH 5.0) to form an insoluble Zn^{2+}−coffee complex and left for 12 h at 4 °C. Each complex was separated by centrifugation (1000*g*, 13 min), then dissolved in 10 mL of a 1% aqueous ammonium solution (sample A), and successively passed through ion-exchange columns (1.5ϕ × 30 cm) of Amberlite IRA-410 [OH^-] (Japan Organo, Tokyo, Japan) and Amberlite IR-120B [NH_4^+] (Japan Organo, Tokyo, Japan). The nonadsorbed fraction was lyophilized (sample Ap), applied to a cellulose column (1.5ϕ × 30 cm), and eluted with mixtures of 2-propanol and a 1% aqueous ammonia solution (5:2, 250 mL; 3:2, 125 mL; 1:1, 150 mL; v/v). The degree of browning (OD_{470}) and Zn concentration of each eluate were measured. The Zn concentration was determined by an AA-670 atomic absorption spectrometer (Shimadzu, Kyoto, Japan). The active fractions (ApV) were collected, lyophilized, and used for further analyses. ApVs obtained from light-, medium-, and dark-roasted coffee were dissolved in water to a concentration of 1%. The absorbance at 470 nm and the Zn content of 1% ApV were measured.

Milk-added ApV was prepared from 10 mL of milk (fats, 3.9%; carbohydrates, 4.95%; proteins, 3.4%; others, 0.3%; w/v; Meiji, Tokyo, Japan) containing a 10 mM hexamethylenetetramine buffer solution (pH 5.0) to which was added 10 g/20 mL instant coffee containing the 10 mM hexamethylenetetramine buffer solution (pH 5.0). The components were then mixed and left for 10 min at room temperature. To the mixture was added 0.082 g of $ZnCl_2$, which was left for 12 h at 4 °C, before the mixture was purified by the same procedure as that used for the ApV of instant coffee.

Determination of Polyphenol Content. The polyphenol content in ApV was estimated by the Folin−Denis method (*18*) and is expressed as microgram equivalents of chlorogenic acid per milligram. ApV was dissolved in water to a concentration of 0.1 mg/mL.

Amino Acid Analysis. ApV (10 mg) was hydrolyzed with 6 N HCl (1 mL) at 110 °C for 24 h. The hydrolysate was concentrated and dried in vacuo before being dissolved in 0.01 N HCl (10 mL) and measured with an 835 amino acid analyzer (Hitachi, Tokyo, Japan) after the ninhydrin reaction.

Evaluation of Molecular Weight of ApV. The molecular weight of ApV was evaluated by gel permeation chromatography (GPC) with a molecular weight marker of poly(ethylene glycol). ApV was dissolved in a 50 mM phosphate buffer solution (pH 7.2) and chromatographed by using a precolumn of TSK PWH (7.5 mm ϕ × 75 mm; Tosoh, Tokyo, Japan) and separation column of TSK-gel G-3000PW (7.5 mm ϕ × 600 mm; Tosoh, Tokyo, Japan) with an L-6000 pump (Hitachi, Tokyo, Japan). The eluent was a 50 mM phosphate buffer (KH_2PO_4−Na_2HPO_4, pH 7.2) at a flow rate of 1.0 mL/min, and the eluate was detected by monitoring its absorbance at 280 nm.

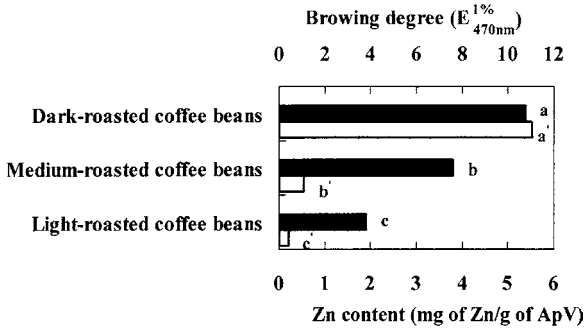

Figure 1. Zinc content (□) and degree of browning (■) of each ApV. Each value is presented as the mean ± SD (n = 3). Different letters show significant differences (a–c, $p < 0.05$; a′–c′, $p < 0.05$).

Evaluation of Chelating Activity. The chelating activity of ApV was evaluated by the tetramethyl murexide (TMM) method (*19*). The sample and reagent were dissolved in a freshly prepared 10 mM hexamethylenetetramine·HCl buffer (pH 5.0). Two milliliters of the sample solution (0.05%, w/v), 0.2 mL of the TMM solution (10 mM), and 2.0 mL of a $ZnCl_2$ solution (from 20 to 100 μM) were mixed. The OD_{460} and OD_{530} values were measured after 30 min, and the free zinc concentration was calculated by using a standard curve. The total zinc concentration was determined by the atomic absorption method, and the percentage of bound zinc was calculated. The amount of bound zinc (millimoles per gram sample) was used to estimate the specific activity of chelation; the total activity was defined as the specific activity multiplied by the sample weight. The number of Zn^{2+}-binding sites (n) and the dissociation constants (K_d) of ApV were determined by Scatchard plot (*20*) data analysis.

Determination of Reducing Activity. The reducing activity of ApV was determined by the indophenol method and is expressed as microgram equivalents of ascorbic acid per milligram. ApV was dissolved in water at a concentration of 1.0 mg/mL.

ABTS Method. The ABTS radical-scavenging assay was conducted according to the method of Pellegrini et al. (*21*). Each sample solution (0.1 mL) was mixed with 2.9 mL of a 75 mM phosphate buffer (pH 7.0) and 1 mL of the ABTS solution (88 μM final concentration). The final concentration of a sample was 25 μg/mL. The test solution was mixed for 30 s, and the absorbance at 734 nm was measured at 30 °C after precisely 3 min. Appropriate blank solvents were run in each assay, and the percentage inhibition was calculated for the blank absorbance at 734 nm. Caffeic acid (0.5, 1.0, 2.0, 4.0, and 6.0 μg/mL final concentration) was used as a positive control. The activity of a sample was expressed as microgram equivalents of caffeic acid per milligram.

Statistical Analysis. Each experiment was run at least three times, with the mean value being shown. A statistical analysis on each sample (ANOVA) was conducted by using StatView 5.0. A difference at $p < 0.05$ is considered significant.

RESULTS AND DISCUSSION

Preparation of the Zinc-Chelating Substance from Coffee. **Figure 1** shows the content of zinc and degree of browning ($E^{1\%}_{470\,nm}$) for each ApV. As the intensity of roasting increased with respect to light-, medium-, and dark-roasted coffee, the Zn content increased and the color of ApV became darker. **Figure 2** shows the yield for ApVs from green and light-, medium-, and dark-roasted coffee. The respective yields of ApVs obtained from green and light-, medium-, and dark-roasted coffee were 0.3%, 0.2%, 0.4%, and 0.6% (w/w). Increasing degree of roasting increased the yield of ApV, apart from that for green coffee beans, suggesting that the amount of melanoidins was directly correlated with the degree of roasting. Borrelli

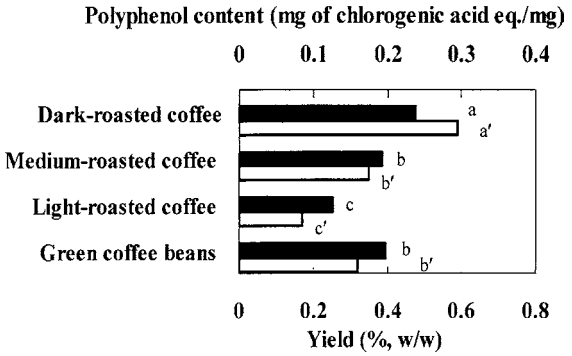

Figure 2. Yield (□) and polyphenol content (■) of each ApV. Each value is presented as the mean ± SD ($n = 3$). Different letters indicate significant differences (a–c, $p < 0.05$; a′–c′, $p < 0.05$).

Table 1. Amino Acid Composition (%, w/w) of ApVs

amino acid	green coffee beans	light-roasted coffee	medium-roasted coffee	dark-roasted coffee
aspartic acid	1.0	1.1	3.8	3.0
threonine	0.4	0.4	0.8	0.2
serine	0.4	0.6	0.4	0
glutamic acid	2.4	2.9	10.4	7.4
glycine	1.4	1.1	2.5	2.2
alanine	0.4	0.5	1.8	1.7
valine	0.4	0.6	2.2	2.1
methionine	0.2	0.1	0.1	0
leucine	0.9	1.3	4.2	3.8
tyrosine	0.2	0	0.9	0.7
phenylalanine	0.4	0.6	2.3	2.1
lysine	0.3	0.7	0.6	0.6
histidine	0.3	0.2	0.7	0.6
arginine	0.7	0.4	0.10	2.2
total	9.4	9.8	30.7	26.6

et al. similarly found that melanoidin formation was in parallel with a thermal treatment (*2*).

Polyphenol Content. Figure 2 shows the polyphenol content for ApVs from green and light-, medium-, and dark-roasted coffee. The polyphenol content of ApV calculated as chlorogenic acid also increased with roasting (19.7%, 12.6%, 19.3% and 23.9% for green and light-, medium-, and dark-roasted coffee, respectively). However, such phenolic compounds as caffeic acid, chlorogenic acid, and furulic acid are to be found in greater amounts in green coffee beans, while significantly decreasing by up to 50−90% during roasting (*22*, *23*). The value for the polyphenol content of ApV might have increased with increasing melanoidins during roasting. In fact, ApV from dark-roasted coffee showed the greatest reducing activity. Although we used the Folin−Denis method for estimating the polyphenol content, this method is affected by coupounds with reducing activity such as melanoidins.

Contents of Amino Acid Residues. The results of amino acid analyses are shown in **Table 1**. The contents of amino acid residues calculated from the analytical results for ApVs from green and light-, medium-, and dark-roasted coffee were about 9.4%, 9.8%, 30.7%, and 26.6% respectively. The contents of amino acid residues in ApVs from medium- and dark-roasted coffee were higher than those in ApVs from light-roasted and green coffee. This suggests that proteins were decomposed during roasting, and that decomposed proteins were involved in the formation of ApV. Protein breakdown into small fragments during coffee processing is a well-known phenomenon (*24*). The increase in nitrogen content of ApVs indicates

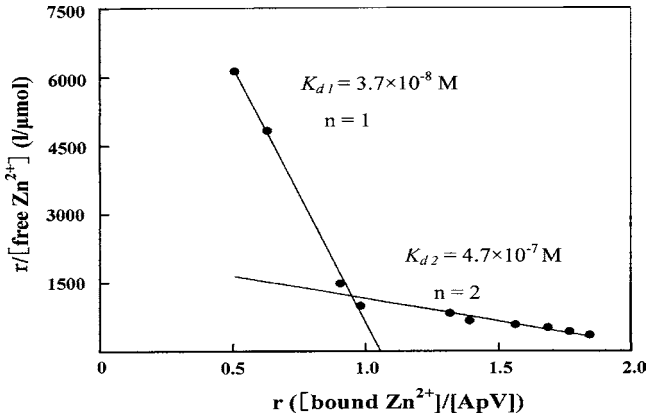

Figure 3. Scatchard plot and approximate lines of ApV obtained from light-roasted coffee. *n*, number of Zn^{2+}-binding sites; K_d, dissociation constant

Table 2. Dissociation Constant, Number of Zn^{2+}-Binding Sites, Molecular Weight, and Molecular Size of the Chelation Unit of Each ApV

ApV of sample	dissociation const, K_d (M)	no Zn^{2+}-binding sites	mol weight	mol size chelation unit[a]
green coffee beans	3.9×10^{-8}	1	28 000	
	4.7×10^{-6}	1.5	28 000	
light-roasted coffee	3.7×10^{-8}	1	29 000	29 000
	4.7×10^{-7}	2	29 000	14 500
medium-roasted coffee	5.8×10^{-7}	3	38 000	12 700
	3.0×10^{-6}	5	38 000	7 600
dark-roasted coffee	2.1×10^{-5}	12	50 000	4 200
instant coffee, no milk	3.4×10^{-7}	3	35 000	
	3.8×10^{-6}	4	35 000	
instant coffee + milk	2.6×10^{-7}	0.5	28 000	
	6.6×10^{-6}	1.5	28 000	

[a] Molecular weight of ApV divided by the number of Zn^{2+}-binding sites.

the involvement of the Maillard reaction in the formation of the Zn-chelating substance in coffee brews.

Zinc-Chelating Activity. Figure 3 shows the Scatchard plot of ApV from light-roasted coffee as an example, from which the numbers of Zn^{2+}-binding sites and the dissociation constants of ApV were calculated. The Scatchard plot shows that ApV of light-roasted coffee had two kinds of Zn^{2+}-binding sites, one strong and the other weak, and the number of weak Zn^{2+}-binding sites ($n = 2$) was higher than that of the strong sites ($n = 1$). The dissociation constants of ApV were $K_{d1} = 3.7 \times 10^{-8}$ (M) and $K_{d2} = 4.7 \times 10^{-7}$ (M). These results indicate that ApV from light-roasted coffee had a high affinity for zinc as citric acid, which is thought to be the main zinc chelator in instant coffee, and had 13% of the total chelating activity of instant coffee (*19*). ApV also contributed to the chelating activity of the brewed coffee. The chelating activities of the other samples were calculated by the same method.

The dissociation constants, the number of Zn^{2+}-binding sites, and the molecular weights of ApVs from light-, medium-, and dark-roasted coffee, green coffee beans, instant coffee, and instant coffee with milk added are shown in **Table 2**. ApV from light-roasted coffee had the highest chelating activity, followed by ApVs from medium- and dark-roasted coffee. With increasing degree of roasting, the dissociation constants of ApV increased from 10^{-8} to 10^{-5}, i.e., the binding strength to Zn^{2+} became weaker, and the number of weak Zn^{2+}-binding sites increased. These results suggest that, as the degree of roasting

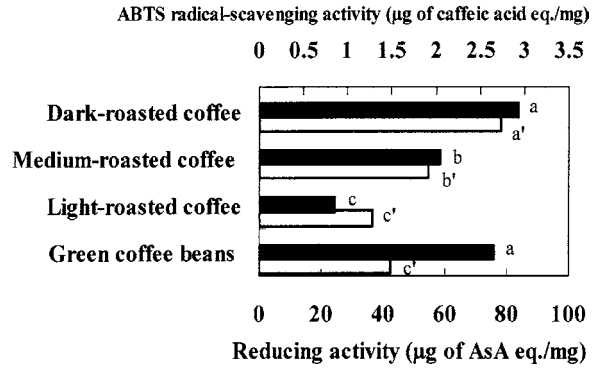

Figure 4. ABTS radical-scavenging (■) and reducing activities (□) of each ApV. Each value is presented as the mean ± SD ($n = 3$). Different letters show significant differences (a–c, $p < 0.05$; a′–c′, $p < 0.05$).

increased, the strong Zn^{2+}-binding sites in ApV were decomposed to the weaker Zn^{2+}-binding sites, and that the dissociation constants and overall number of Zn^{2+}-binding sites increased. Furthermore, it was noted that the $E^{1\%}_{470\,nm}$ value for ApV increased with increasing degree of roasting (**Figure 1**), which suggests that the degree of polymerization and the molecular weight of ApV determined by GPC (**Table 2**) also increased with increasing degree of roasting.

The molecular weight of ApV divided by the number of Zn^{2+}-binding sites (**Table 2**) enabled us to predict the molecular size of the chelation unit in each ApV. The lower the value of K_d, the larger the chelation unit, which shows that the larger the chelation unit, the stronger the zinc-chelating activity. It also suggests that roasting decreases the Zn-chelation unit which binds one zinc molecule in ApV.

Comparing the metal-chelating activities between model melanoidins (K_d, 1.2−5.6 μM) obtained from glucose−glycine (*25*) and the metal-chelating substance, ApV, in coffee brews (K_d, 10^{-2}−10^{-5} μM, **Table 2**), the metal-chelating activity of ApV was much higher than that of melanoidin. This may be due to the existence of phenolic residues in ApV (*13*). Moreover, the dissociation constants of ApV in coffee brews ranged from 10^{-8} to 10^{-5} M, suggesting that the intake of brewed coffee may not have an effect on mineral nutrition absorption in general dietary life and the brown pigments of coffee brews may act as dietary fibers.

ABTS Radical-scavenging and Reducing Activities. Figure 4 shows the ABTS radical-scavenging and reducing activities of ApVs from green and light-, medium-, and dark-roasted coffee. As the intensity of roasting increased, the ABTS radical-scavenging and reducing activities of ApV increased. The reducing activities of ApVs from light-, medium-, and dark-roasted coffee were respectively 6, 12, and 22 times higher than those of ascorbic acid. Enediol and enaminol in melanoidin are involved in the reducing activities that occur in a food system (*26*). With increasing degree of roasting, the melanoidins in ApV increased, and so the reducing activities also increased. As absorbance at 470 nm and the reducing activity increased with increasing degree of roasting, the development of color was probably an index of reducing activity of coffee brews.

The antioxidative activity of ApV from green coffee beans decreased markedly by a light roasting, and then the antioxidant activity of ApV increased with roasting (**Figure 4**), suggesting that, although OH groups with antioxidant activity in ApV were lost during roasting, the overall antioxidant activities of ApV could be maintained, or even enhanced, by the development of compounds that possess antioxidant activity, including the Maillard reaction products.

Richelle et al. (*27*) reported that the antioxidant activity of coffee decreased with roasting, whereas in other studies a maximum antioxidant activity at medium-roasted coffee was observed (*28*, *29*). Relationships between degree of roasting and antioxidant activity vary according to the type of coffee, roasting conditions, extraction procedure, and antioxidant assay (*30*). In contrast with these results (*27*−*29*), the antioxidative activity of ApV increased with roasting. The samples used in the above-mentioned literature (*27*−*29*) were coffee extracts, which contained low-molecular-weight phenolic compounds and Maillard reaction products, whereas ApV is a high-molecular-weight substance with a partial structure similar to those of melanoidin and polymers of phenolic compounds in coffee brews, and was purified from the coffee extracts. The antioxidative activity of ApV is due not only to the presence of phenolic residues in ApV but also, more importantly, to the presence of Maillard reaction products generated during roasting. The proton-donating or reducing activities and chelating activity appear to be important for the antioxidative activity of ApV.

Relationship between the Chelating and Reducing Activities of ApV. A negative correlation between the chelating activity and the reducing or antioxidative activitiy was apparent. Three main effects of roasting coffee beans are likely to be protein decomposition (*24*), the formation of melanoidins, and the degradation and polymerization of low-molecular-weight phenolic compounds (*31*, *32*). Our previous data showed that ApV from instant coffee contained about 10−20% phenolics, which was detemined by the alkali fusion of ApV and HPLC method, 10.5% nitrogen, and only 3−4% amino acid residues and sugars (*13*). The zinc-chelating substance in coffee, ApV, was considered to be a melanoidin-like polymer formed by decomposition and polymerization of sugars, amino acids, and phenolics, and its metal-chelating activity may be mainly due to phenolic residues (*6*, *13*). Free OH groups of phenol residues in ApV seem to be decreased during roasting. Therefore, the zinc-chelating activity of ApV decreased with roasting (**Table 2**). In contrast, as the intensity of roasting increased, the reducing and antioxidative activities of ApV simultaneously increased, which was in agreement with the increase of melanoidins during roasting. These results suggest that, as phenolic OH groups in ApV decreased during thermal treatments, melanoidins probably became the prevailing contributors to the reducing and antioxidative activities of ApV.

Effect on the Zinc-Chelating Activity of Adding Milk to Brewed Coffee. After milk had been added to instant coffee and its ApV prepared, the number of Zn^{2+}-binding sites and the molecular weight of ApV decreased, but the dissociation constants remained almost unchanged. As ApV is a chelating substance, it would combine with Ca^{2+} in milk, and the number of Zn^{2+}-binding sites might decrease. Alternatively, the three-dimensional structure of ApV might change when milk is added to instant coffee, leading to a decrease in the number of Zn^{2+}-binding sites. However, adding milk to instant coffee may not cause any structural change in the Zn^{2+}-binding sites, because the dissociation constants for ApV remained almost unchanged. The molecular weight of ApV from instant coffee with added milk was lower than that of ApV from nonadded instant coffee, suggesting that some part of ApV had been removed by adding milk to instant coffee.

Coffee brews are often consumed with milk; therefore it seems to be necessary to evaluate the metal-chelating activity of coffee brews in the presence of milk. We found that the dissociation constants of ApV remained almost unchanged by the presence of milk, suggesting that addition of milk did not

alter the zinc-chelating activity of ApV, and the number of Zn^{2+}-binding sites of ApV decreased, indicating that adding milk to brewed coffee to drink may have a favorable effect on zinc absorption. It has been reported that milk and vegetable products added to coffee beverages affected the release of aroma substances from the brew through their lipid, protein, and carbohydrate components (*15*, *16*). Recently, it has been shown that the milk proteins inhibit the antioxidant activity of teas (*33*). In contrast, Richelle et al. have reported that the antioxidant activities of coffee, cocoa, and black tea were not affected by the presence of milk (*27*).

In conclusion, the zinc-chelating activity of brewed coffee decreased as the intensity of roasting was increased. The metal-chelating activity of ApV may be mainly due to phenolic residues. The reducing activities of ApVs were stronger those that of ascorbic acid, and as the intensity of roasting was increased, the reducing and antioxidative activities of ApV were increased. The weaker the chelating activity, the stronger the reducing activity. Melanoidins were the main contributor to the antioxidative and reducing activities of Zn-chelating substances in brewed coffee. Milk added to instant coffee decreased the number of Zn^{2+}-binding sites of ApV, but the zinc-chelating activity of ApV was not altered.

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LITERATURE CITED

(1) Clifford, M. N. Chlorogenic acids. In *Coffee, Chemistry*; Clarke, R. J., Macrae, R., Eds.; Elsevier Applied Science Press: London, 1985; Vol. 1, pp 239−241.

(2) Borrelli, R. C.; Visconti, A.; Mennella, C.; Anese, M.; Fogliano, V. Chemical characterization and antioxidant properties of coffee melanoidins. *J. Agric. Food Chem.* **2002**, *50*, 6527−6533.

(3) Okada, H.; Murata, M.; Ohyazu, K.; Homma, S. Distinction between arabica and robusta coffee beans by hydroxycinnamic acid derivatives, especially by *p*-coumaroyl-(L)-tryptophan. *Food Sci. Technol.* **1997**, *3*, 205−209.

(4) Nakabayashi, T.; Watanabe, C. Chemical studies on the quality of coffee, part IV. Formation of brown pigments from chlorogenic acid by roasting. *Nippon Shokuhin Kogyo Gakkaishi* **1975**, *24*, 124−129 (in Japanese).

(5) Maier, H. G.; Buttle, H. Isolation and characterization of brown roast compounds of coffee. *Z. Lebensm.-Unters. Forsch.* **1973**, *150*, 333−334 (in German).

(6) Wen, X.; Takenaka, M.; Murata, M.; Homma, S. Antioxidative activity of a zinc-chelating substance in coffee. *Biosci., Biotechnol., Biochem.* **2004**, *68* (11), 2313−2318.

(7) Mork, T. A.; Lynch, S. R.; Cook, J. D. Inhibition of food iron absorption by coffee. *Am. J. Clin. Nutr.* **1983**, *37*, 416−420.

(8) Gomyo, T.; Horikoshi, M. On the interaction of melanoidin with metallic ions. *Agric. Biol. Chem.* **1976**, *40*, 33−40.

(9) Morales, F. J.; Babbel, M. B. Antiradical efficiency of Maillard reaction mixture in hydrophilic media. *J. Agric. Food Chem.* **2002**, *50*, 2788−2792.

(10) Rendleman, J. A., Jr. Complexation of calcium by melanoidin and its role in determining bioavailability. *J. Food Sci.* **1987**, *52*, 1699−1705.

(11) Murray, L. W.; Connie, M. W. Maillard browning effects on *in vitro* availability of zinc. *J. Food Sci.* **1988**, *53*, 1508−1510.

(12) Fukushima, M.; Tanimura, A. Binding property of aluminium ion to dietary fiber, polyphenol compounds and organic acids. *Nippon Shokuhin Kagaku Kogaku Kaishi* **1995**, *42*, 425−429 (in Japanese).

(13) Takenaka, M.; Sato, N.; Asakawa, H.; Wen, X.; Murata, M.; Homma, S. Characterization of a metal-chelating substance in coffee. *Biosci., Biotechnol., Biochem.* **2005**, *69* (1), 26−30.

(14) Homma, S.; Murata, M. Characterization of metal-chelating compounds in instant coffee. In *16th International Scientific Colloquium on Coffee (Kyoto)*; ASIC, Paris, France, 1995; pp 183−191.

(15) Kim, J. K.; Rho, J.; Kim, S. Y. The effect of creamer/milk addition to coffee aroma retention in coffee solution. In *16th International Scientific Colloquium on Coffee (Kyoto)*; ASIC, Paris, France, 1995; pp 164−173.

(16) Bucking, M.; Steinhart, H. Headspace GC and sensory analysis characterization of the influence of different milk additives on the flavor release of coffee beverages. *J. Agric. Food Chem.* **2002**, *50*, 1529−1534.

(17) Daglia, M.; Cuzzoni, M. T.; Dacarro, C. Antibacterial activity of coffee. *J. Agric. Food Chem.* **1994**, *42*, 2270−2272.

(18) Singleton, V. L.; Rossi, J. A. Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. *Am. J. Enol. Vitic.* **1965**, *16*, 144−158.

(19) Nakamura-Takada, Y.; Shata, H.; Minao, M.; Ogawa, H.; Sekiguchi, N.; Murata, M.; Homma, S. Isolation of a zinc-chelating compound from instant coffee by the tetramethyl murexide method. *Lebensm.-Wiss. Technol.* **1994**, *27*, 115−118.

(20) Kohn, R.; Furda, I. Calcium ion activity in solutions of calcium pectinate. *Collect. Czech. Chem. Commun.* **1967**, *32* (5), 1925−1937.

(21) Pellegrini, N.; Re, R.; Yang, M.; Evans, C. R. Screening of dietary carotenoids and carotenoid-rich fruit extracts for antioxidant activities applying 2,2′-azinobis-ethylenebenzothiazoline-6-sulfonic acid radical cation decolorization assay. *Methods Enzymol.* **1999**, *299*, 379−389.

(22) Stich, H. F. The beneficial and hazardous effects of simple phenolic compounds. *Mutat. Res.* **1991**, *259*, 307−324.

(23) Clifford, M. N. Chlorogenic acids: Their complex nature and routine determination in coffee beans. *Food Chem.* **1979**, *4*, 63−71.

(24) Macrae, R. Nitrogenous compounds. In *Coffee, Chemistry*; Clarke, R. J., Macrae, R., Eds.; Elsevier Applied Science Press: London, 1985; Vol. 1, pp 115−151.

(25) Terasawa, N.; Murata, M.; Homma, S. Separation of model melanoidin into components with copper chelating Sepharose 6B column chromatography and comparison of chelating activity. *Agric. Biol. Chem.* **1991**, *55* (6), 1507−1514.

(26) Homma, S.; Terasawa, N.; Kubo, T.; Yoneyama, I. N.; Aida, K.; Fujimaki, M. Changes in chemical properties of melanoidin by oxidation and reduction. *Biosci., Biotechnol., Biochem.* **1997**, *611* (3), 533−535.

(27) Richelle, M.; Tavazzi, I.; Offord, E. Comparison of the antioxidant activity of commonly consumed polyphenolic beverages (coffee, cocoa, and tea) prepared per cup serving. *J. Agric. Food Chem.* **2001**, *49*, 3438−3442.

(28) Nicoli, M. C.; Anese, M.; Manzocco, L.; Lerici, C. R. Antioxidant properties of coffee brews in relation to the roasting degree. *Lebensm.-Wiss. Technol.* **1997**, *30*, 292−297.

(29) Castillo, M. D.; Ames, J. M.; Gordon, M. H. Effect of roasting on the antioxidant activity of coffee brews. *J. Agric. Food Chem.* **2002**, *50*, 3698−3703.

(30) Schwarz, K.; Bertelsen, G.; Nissen, L. R.; Gardner, P. T.; Heinonen, M. I.; Hopia, A.; Hunh-Ba, T.; Lambelet, P.; McPhail, D.; Skibsted, L. H.; Tijburg, L. Investigation of plant extracts for the protection of processed foods against lipid oxidation. Comparison of antioxidant assays based on radical scavenging, lipid oxidation and analysis of the principal antioxidant compounds. *Eur. Food Res. Technol.* **2001**, *212*, 319−328.

(31) Nunes, M. F.; Coimbra, M. A. Chemical characterization of the high molecular weight material extracted with hot water from green and roasted Arabica coffee. *J. Agric. Food Chem.* **2001**, *49*, 1773−1782.

(32) Parliament, T. H. An overview of coffee roasting. In *Caffeinated beverages: health benefits, physiological effects and chemistry*; Parliament, T. H., Ho, C. T., Schieberle, P., Eds.; ACS Symposium Series 754; American Chemical Society: Washington, DC, 2000; pp 188−201.

(33) Leenen, R.; Roodenburg, A. J.; Tijburg, L. B. M.; Wiseman, S. A. A single dose of tea with or without milk increase plasma antioxidant activity in humans. *Eur. J. Clin. Nutr.* **2000**, *54*, 87−92.

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