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γ-Tocopherol as a Marker of Brazilian Coffee (*Coffea arabica* L.) Adulteration by Corn

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The adulteration of coffee with cereals, coffee twigs, etc. is apparently widespread in Brazil with corn being considered the most widely used. No adequate methods are available to detect such contamination in commercial coffee. A new method, based on high-performance liquid chromatography (HPLC) tocopherol determination was developed to detect coffee adulteration by corn. Percentages of α-, β-, γ-, and δ-tocopherol determined by HPLC in six coffee varieties were 29.0, 61.7, 3.3, and 6.0, respectively. Similar values were obtained in six popular coffee brands. The percentages of α-, γ-, and δ-tocopherol in six corn samples were 3.6, 91.3, and 5.1, respectively. These differences could be applied to detect corn in a pure coffee sample intentionally contaminated with corn with the best result obtained with γ-tocopherol. With this methodology, one coffee brand was apparently adulterated (8.9%), most likely with corn. Tocopherol fingerprinting offers the potential to detect adulteration.

KEYWORDS: Coffee; adulteration; corn; tocopherols

INTRODUCTION

There are several coffee species but only *Coffea arabica* and *Coffea canephora* (*robusta*) or their blends are used to obtain most roasted coffee. *C. arabica* has a higher commercial value than *C. robusta* due to its pronounced flavor, and it is speculated that it is adulterated with lower priced adulterants, that is, cereals, coffee twigs, coffee, brown sugar, etc. (*1*). The Association of the Brazilian Coffee Industry considers adulteration one of the most serious problems affecting Brazilian coffee quality. However, there is no official data since adequate methods to detect contamination have not yet been developed.

Methods to determine food authenticity have recently been reviewed and include spectroscopy (ultraviolet, near and mid-infrared, visible, and Raman spectroscopy), isotopic analysis, chromatography, electronic nose, polymerase chain reaction, enzyme-linked immounosorbent assay, thermal analysis, and chemometrics (*2*). Few studies have been conducted with coffee. Among the methods available for determination of adulteration are digital imaging (*1*, *3*), thermal lens and pH (*4*), infrared spectroscopy/chemometrics (*5*), voltammogrammetry/chemometrics (*6*), microscopy (*7*), and high-performance liquid chromatography (HPLC) (*8*). However, most of these methods are not applied to commercial samples.

Most studies on coffee adulteration have attempted to distinguish *C. arabica* from *C. robusta* using chemical parameters, that is, diterpenic alcohols (*9*), sterols (*10*, *11*), volatiles (*12*), metal content (*13*), chlorogenic acid and caffeine (*14*), fatty acids (*15*, *16*), tocopherols and triglycerides (*17*), and amino acids (*18*).

Several adulterants of widely differing chemical compositions, ranging from the less expensive coffee species (*C. robusta*), cereals (corn, soybean, etc.), grains, carbohydrates, coffee twigs, caramel, etc. are apparently used. This is further complicated by the fact that several varieties of both coffee and adulterants could be used. Environmental and processing effects on the chemical compositions of coffee and adulterants are unknown. Little or no literature on coffee adulteration could also be another limiting factor. Given the complexity of the problem, it was apparent that specific methodology would have to be developed for each adulterant. This would be very time-consuming and tedious. Our short-term goal was to develop methods to determine coffee adulteration, and we chose to initially investigate corn as it is apparently the most widely used due to its significantly lower cost.

In this study, tocopherols were analyzed in six Brazilian coffee (*C. arabica* L.) varieties (Catuaí, Catuca, Burbourn, Mundo Novo, Rubí, and Topázio), six commercial coffee (*C. arabica*) brands, one pure commercial coffee (*C. arabica* L.) sample intentionally adulterated with roasted corn (5, 10, and 20%, w:w, corn:coffee), and six roasted corn samples. On the basis of these values, a new method to detect adulteration of commercial coffee with corn is reported.

MATERIALS AND METHODS

Chemicals. HPLC grade hexane and 2-propanol were purchased from Sigma-Aldrich, Inc. (St. Louis, MO). Tocopherol standards (≥97% purity) were obtained from Matreya, LLC (Pleasant Gap, PA).

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Coffee and Corn Samples. The roasting, grinding, and extraction processes described below were performed in triplicate for each sample and/or variety.

Six Coffee Varieties (Samples 1−6). About 1 kg of six green coffee (*C. arabica*) bean varieties (Catuaí, Catuca, Burbourn, Mundo Novo, Rubí, and Topázio) was supplied by Incofex Inc. (Viçosa, MG, Brazil). The samples were divided into roughly three equal parts. Each part was roasted at 180 °C for 10 min in a coffee roaster with three burners (Jcarmo, Sao Paulo, Brazil, model tp-3) and ground in a coffee grinder (Jcarmo, model RA 23-E).

Commercial Coffee (Samples 16−21). One kilogram of six roasted and ground coffee (*C. arabica*) brands was purchased from a local supermarket in Viçosa, MG, Brazil. All samples were divided into roughly three equal portions.

Intentionally Adulterated Coffee (C. arabica) (Samples 13−15). Commercial coffee (sample 21) was mixed with 5, 10, and 20% (w:w, coffee:corn) of ground roasted corn to yield samples 13−15, respectively.

Commercial Corn (Samples 7−12). One kilogram of six commercial unroasted corn samples was randomly selected from a local supermarket in Viçosa, MG, Brazil. All of the samples were divided into roughly three equal portions, roasted, and ground under the conditions utilized for coffee as previously described.

Oil Extraction. About 10 g of all of the samples was extracted with hexane in a Soxhlet extractor overnight to obtain oils, which were sealed under N_2 and stored in a freezer (−5 °C) until HPLC analysis.

HPLC Analysis. The oils were weighed, diluted in hexane to a concentration of 10−15 mg/mL, and filtered through 0.45 μm centrifugal filters (National Scientific, Rockwood, TN) for HPLC analysis. Tocopherol HPLC analysis was performed according to the AOCS official method Ce 8-89 (*19*). The HPLC system consisted of a Varian (Varian, Inc., Palo Alto, CA) Pro-Star pump, autosampler, and fluorescence detector. The mobile phase consisted of hexane:2-propanol (99.5:0.5 v/v, made fresh daily) pumped at 1 mL/min. Samples were injected by autosampler using the full loop option (100 μL), and tocopherols were separated using an Inertsil (Varian, Inc), silica column (5 μm, 150 Å, 250 mm × 4.6 mm i.d.). The fluorescence detector was set with an excitation wavelength of 290 nm and an emission wavelength of 330 nm. Data collection and integration were performed with Varian Star Chromatography version 6.0. Tocopherol peaks were identified by retention times of known standards. A mixture of α-, β-, γ-, and δ-tocopherol standards was injected on each day of analysis to verify HPLC performance.

Quantification of Tocopherols in Coffee and Corn Samples. Two types of quantification were carried out. In the first, the relative area percentages for α-, β-, γ-, and δ-tocopherol were determined by dividing each peak area by the sum of the tocopherol peak areas and multiplying by 100. In addition, the external standard method was used. Linear standard curves ($R^2 > 0.99$) of concentration vs peak area were generated using duplicate injections of α- (0.5−50 μg/mL), β- (0.2−8 μg/mL), γ- (0.5−40 μg/mL), and δ-tocopherol (0.1−40 μg/mL). The lowest concentration for each tocopherol used in the standard curve was chosen as the lower limit of detection.

Determination of Corn Adulteration in a Commercial Coffee Sample (Sample 18). Quantification of corn in a commercial coffee sample with a higher relative area percentage of γ-tocopherol was obtained from a calibration curve. This curve was constructed by plotting the % (5, 10, and 20, w:w, corn:coffee) of roasted corn added to a noncontaminated roasted commercial coffee sample vs average relative percentage of γ-tocopherol in the intentionally adulterated coffee:corn mixtures.

Statistical Analyses. Data were imported into SAS for Windows version 9.1 (SAS Institute, Inc., Cary, NC) for statistical analysis. The effect of variety, or sample when variety was unknown, on contents (mg/kg) and relative area percentages of α-, β-, γ-, and δ-tocopherols for the different Brazilian *C. arabica* coffee varieties, the Brazilian commercial coffees, the Brazilian corn, and the corn-contaminated coffee samples were compared using one-way analysis of variance. Means were compared using Duncan's multiple range test; the significance was determined by p value < 0.05. Linear regression analysis with area % of α-, β-, or γ-tocopherol as the dependent variable was used to analyze the effect of corn contamination levels on the relative area % of the tocopherols. The parameter estimates generated for γ-tocopherol were used to estimate the level of contamination of corn in one commercial coffee sample (sample #18) that had a significantly higher relative area percentage of γ-tocopherol.

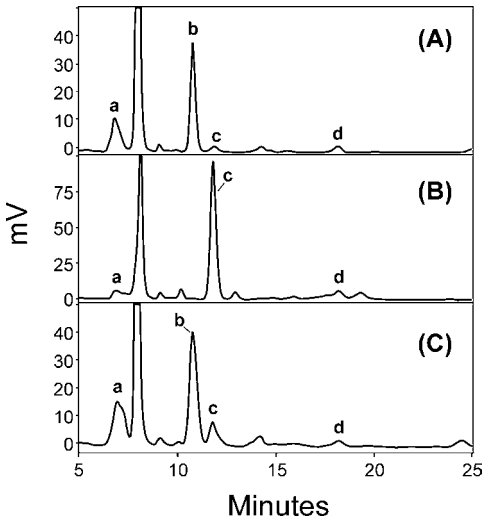

Figure 1. Typical chromatograms obtained on HPLC analysis of crude coffee extracts of the variety Novo Mundo (**A**), roasted corn sample (sample 1) (**B**), and a coffee sample intentionally adulterated with 20% corn (**C**). Peaks are labeled as follows: a, α-tocopherol; b, β-tocopherol; c, γ-tocopherol; and d, δ-tocopherol.

Table 1. Relative Area % of Tocopherols in Oils from Six Varieties of Brazilian Coffee (*C. arabica* L.)

		area %[a]			
sample	coffee variety	α	β	γ	δ
1	Catuaí	29.8 ± 0.6	61.3 ± 0.5	3.0 ± 0.6	6.0 ± 0.6
2	Catucai	27.9 ± 0.7	63.2 ± 0.5	3.9 ± 0.1	5.0 ± 0.2
3	Burbourn	28.8 ± 0.4	61.3 ± 0.8	3.7 ± 0.3	6.3 ± 0.6
4	Mundo Novo	27.6 ± 2.1	62.9 ± 3.2	4.3 ± 0.5	5.3 ± 1.6
5	Rubí	29.2 ± 0.5	61.5 ± 0.2	3.7 ± 0.5	5.6 ± 0.2
6	Topázio	29.3 ± 2.6	61.1 ± 1.6	2.4 ± 1.4	7.2 ± 0.4
	average	29.0 ± 1.5	61.7 ± 1.5	3.3 ± 1.1	6.0 ± 1.0
	P value[b]	0.2448	0.6236	0.0152	0.2078

[a] Values are averages of three determinations. [b] *P* value for the *F* test determination of the significance of variety on tocopherol area %; $p < 0.05$ is significant.

RESULTS AND DISCUSSION

Despite complex chemical coffee compositions (*20*), typical HPLC traces obtained on analysis of crude coffee (**Figure 1A**) and corn (**Figure 1B**) extracts were quite simple. Under these conditions, the four tocopherols eluted before 20 min. There are often problems separating β- and γ-tocopherol, but under these conditions, they were almost completely baseline resolved at 10.8 and 11.8 min, respectively. There was a large peak present in all samples, including both roasted coffee and corn, which eluted immediately after α-tocopherol but was sufficiently resolved that it did not interfere with quantitation. As it can be seen from **Figure 1A**,**B**, significant differences were observed between coffee and corn tocopherols, which were used to develop a new methodology to detect corn adulteration in coffee.

Tocopherol Content of Six Brazilian Coffee Varieties. The relative area percentages of tocopherols in the six roasted Brazilian coffee varieties are presented in **Table 1**. Relative area percentages of α-, β-, γ-, and δ- tocopherols of Catuaí, Catuca,

Table 2. Relative Area % of Tocopherols in Oils from Six Roasted Commercial Corn Samples

	area %[a]		
sample	α	γ	δ
7	4.7 ± 1.7	90.2 ± 0.8	5.1 ± 0.8
8	4.6 ± 1.3	90.2 ± 1.6	5.2 ± 0.5
9	3.3 ± 0.0	91.4 ± 0.3	5.3 ± 0.3
10	3.3 ± 0.2	91.5 ± 0.1	5.2 ± 0.1
11	3.0 ± 0.1	91.6 ± 0.9	5.5 ± 0.9
12	3.0 ± 0.2	92.5 ± 0.5	4.5 ± 0.4
average	3.6 ± 0.9	91.3 ± 1.1	5.1 ± 0.5
P value[b]	0.0697	0.0655	0.4315

[a] Values are averages of three determinations. [b] *P* value for the *F* test determination of the significance of variety on tocopherol area % or content (mg/kg); $p < 0.05$ is significant.

Burbourn, Mundo Novo, Rubí, and Topázio varied between 27.6 and 29.8, 61.1 and 63.2, 2.4 and 4.3, and 5.3 and 7.2, respectively (**Table 1**). In all cases, the area percentage of the tocopherols decreased as follows: β > α > δ > γ. The concentrations of α-, β-, γ-, and δ-tocopherol in the coffee oils varied between 373 and 475, 491 and 659, 34.6 and 61.9, and 24.6 and 31.7 mg/kg, respectively (data not shown). In all varieties, the concentration of the tocopherols decreased as follows: β > α > γ > δ. This order was reversed for δ- and γ- tocopherols when absolute concentrations were considered because the response factor (i.e., the slope of the standard curve) was higher for δ- as compared to γ-tocopherol. The relatively high contents of α- and β-tocopherol and low γ- and δ-tocopherol contents in coffee have been verified by several other studies. Values ranging between 89 and 188 mg/kg of α-tocopherol and 252−530 mg/kg of β- and γ-tocopherols (these two were unresolved by thin-layer chromatography or gas chromatography) were reported in oil extracted from green coffee beans of both *C. arabica* and *C. robusta* varieties (*21*). Tocopherols in green and roasted coffee beans from 14 countries and in 43 different instant coffees presented α- and β-tocopherol ratios similar to our study, but γ- or δ-tocopherols were not detected (*22*). Ratios of α-, β-, and γ-tocopherols in different varieties of coffee beans were approximately 2:4:0.1 with no δ-tocopherol (*23*). Tocopherols in coffee from several countries were reported with β > α > γ-tocopherol contents in green *C. arabica* and *C. robusta* beans but found that γ-tocopherol increased to ratios higher than α-tocopherol in roasted beans (*17*). The dramatic increase in γ-tocopherol content in roasted coffee beans in that study is confounding since no other studies have reported such high concentrations of γ-tocopherol. In many of the above studies, the coffee variety was not specified.

There was no significant effect of variety on the relative area percentages of α-, β-, and δ-tocopherols, indicating that the tocopherol profile is relatively constant among these varieties of Brazilian coffee. There was a significant effect of variety on the relative percentage of γ-tocopherol, mainly due to the lower area percentage in the Topázio variety. Among all varieties, the average percentage of γ-tocopherol was 3.3 ± 1.1%.

Tocopherol Content of Six Roasted Brazilian Corn Samples. The relative area percentages of tocopherols in the six roasted Brazilian corn samples are presented in **Table 2**. In all samples, γ-tocopherol was the major component, accounting for about 90% of the total tocopherols, and in all samples, the concentration of tocopherols decreased as follows: γ > δ > α. The concentration of α-, γ-, and δ- tocopherols for the six corn samples varied from 65.3 to 112, 905 to 1124, and 29.5 to 41.0 mg/kg, respectively (data not shown). Once again, absolute ratios of tocopherols changed (γ > α > δ) as compared to the relative percentages because δ-tocopherol had a higher response factor than the other tocopherols. The tocopherol profiles of these corn samples are in agreement with other studies of tocopherols obtained from hexane-extracted corn kernels (*24*) and are also similar to commercial corn oil tocopherol composition (*25*). Corn sample did not have a significant effect ($p < 0.05$) on tocopherol relative area percentages.

Table 3. Area % of Tocopherols in a Commercial Brazilian Coffee (*C. arabica* L.) (Sample 21) Intentionally Contaminated with Different Amounts of Roasted Corn

	composition (%, w/w)		area %[a]			
sample	coffee	corn	α	β	γ	δ
21	100	0	31.4 ± 0.2	63.3 ± 1.3	2.5 ± 1.6	2.8 ± 0.3
13	95	5	32.0 ± 0.6	59.6 ± 1.0	6.0 ± 0.1	2.4 ± 0.7
14	90	10	30.7 ± 0.2	60.1 ± 0.3	7.6 ± 0.2	1.6 ± 0.1
15	80	20	27.8 ± 1.3	57.6 ± 0.3	10.4 ± 0.9	4.2 ± 0.4
	P value[b]		0.0004	0.0003	0.0001	0.0005

[a] Values are averages of three determinations. [b] *P* value for the *F* test determination of the significance of corn contamination on tocopherol area %; $p < 0.05$ is significant.

Tocopherol Content in Coffee Contaminated with Corn. The results described above indicate that there are major differences between coffee and corn in tocopherol profiles as well as contents of individual tocopherols, especially in γ-tocopherol. To verify whether it would be possible to detect corn in coffee, we intentionally adulterated a pure commercial coffee sample (sample 21) with ground, roasted corn. The % contamination was arbitrarily chosen and based on the supposition that it had to be sufficiently high for a financial return but at the same time could not be too high as to significantly alter the coffee's flavor. As we have seen, tocopherol content is affected by variety in both coffee and corn, and tocopherols are also susceptible to loss through oxidation and heat (*26*); thus, the tocopherol content in coffee could also be influenced by processing and storage parameters. Therefore, we determined that the most straightforward method for detecting corn contamination would be to focus on changes in the tocopherol peak area percentages rather than on total tocopherol content. The data obtained with intentional adulteration of 5, 10, and 20% roasted corn are presented in **Table 3** along with a representative chromatogram in **Figure 1C**. The chromatogram shows an increase in γ-tocopherol as compared to pure coffee samples. As expected, changes in relative tocopherol percentage were recorded with the four tocopherols. For α-tocopherol, no significant differences ($p < 0.05$) in relative tocopherol percentage were recorded between the samples intentionally contaminated with 5 and 10% roasted corn from the original (uncontaminated) coffee. However, coffee contaminated with 20% corn was significantly ($p < 0.05$) lower in α-tocopherol, which is what might be expected since corn had a lower absolute and relative α-tocopherol as compared to coffee. For β-tocopherol, the samples contaminated with 5 and 10% corn were significantly lower than the original, and the sample contaminated with 20% corn was significantly lower than all three. This is also as would be expected, since no β-tocopherol was detected in the roasted corn. The percentage of γ-tocopherol, as expected, increased significantly in the contaminated samples as compared to the original, with no difference between 5 and 10% levels of contamination but a significant increase at the 20% level. There was no pattern of change in levels of δ-tocopherol with corn contamination, most likely because both absolute and relative

Table 4. Relative Area % of Tocopherols in Six Commercial Brazilian Coffee (*C. arabica* L.) Samples

	area %[a]			
sample	α	β	γ	δ
16	32.5 ± 1.3 B	62.7 ± 1.1	2.2 ± 0.3	2.6 ± 0.2
17	34.1 ± 2.2 B	62.1 ± 2.2	2.2 ± 0.4	1.6 ± 0.3
18	39.4 ± 1.4 A	50.6 ± 2.7	6.7 ± 2.8	3.4 ± 0.8
19	33.0 ± 1.8 B	61.3 ± 2.7	2.4 ± 1.8	3.3 ± 1.1
20	27.7 ± 1.9 C	66.9 ± 1.9	3.1 ± 1.3	2.3 ± 0.6
21	31.4 ± 0.2 B	63.3 ± 1.3	2.5 ± 1.6	2.8 ± 0.3
average	33.0 ± 3.8	61.2 ± 5.5	3.2 ± 2.1	2.7 ± 0.8
P value[b]	<0.0001	<0.0001	0.0361	0.0463

[a] Values are averages of three determinations. [b] *P* value for the *F* test determination of the significance of sample on tocopherol area % or content (mg/kg); $p < 0.05$ is significant.

percentage levels of this tocopherol are similar in coffee and corn. Linear regression analysis of α-, β-, and γ-tocopherol changes in response to corn contamination all resulted in significant parameter estimates (data not shown), but the best response was obtained with γ-tocopherol. The linear increase obtained with the increase in the % of corn added could be described by the equation: $Y = 3.30 + 0.378 \times X$, where Y is the relative area percentage for γ-tocopherol and X is the level of corn contamination. The standard error for the intercept and the slope are 0.49 and 0.04, respectively, and the correlation coefficient (R^2) is 0.8848. Hence, it appears that γ-tocopherol fingerprinting presents the potential as a marker to detect corn adulteration.

Tocopherol Content in Six Commercial Brazilian Coffees. The tocopherol composition of the six commercial Brazilian coffees is presented in **Table 4**. Relative area percentages of the four tocopherols in most samples were very similar to those seen in the coffee varieties that were roasted in-house. The average area percentage of α-tocopherol was slightly higher (33.0 ± 3.8) in the commercial samples as compared to the in-house samples, and the δ-tocopherol average was lower. Also, the variability among the commercial samples was higher. This could be related to processing conditions (roasting, storage, handling, etc.) used by different manufacturers. The tocopherol profile showed β > α, while γ- and δ-tocopherols were more similar to each other. Concentrations of α-, β-, γ-, and δ-tocopherols varied between 349 and 825, 455 and 749, 31.9 and 113, and 5.37 and 21.7 mg/kg, respectively (data not shown). The coffee sample had a significant effect ($p < 0.05$) on the concentrations and relative area percentages of α-, β-, and δ-tocopherols. However, concentration and relative area percentage of γ-tocopherol were the same in all but one of the six commercial brands; only sample 18 presented a significantly higher ($p < 0.05$) content and relative area % of γ-tocopherol, as compared to the other samples. This indicates that this sample may be adulterated with a contaminant such as corn that is higher in γ-tocopherol than coffee. On the basis of this value and our linear regression model for corn contamination vs γ-tocopherol levels in coffee, this sample could be considered to be adulterated with 8.9% corn.

Results reported in this study should be considered somewhat preliminary but important. Several factors, that is, variety, sample origin, storage, processing etc., which could affect tocopherols, should be examined in details. These very large numbers of factors forced us to limit our sample size in this study. Despite this limitation, the method described is a significant improvement over the literature methods to detect corn adulteration in coffee because it is simple; the steps involve extracting oil and diluting for HPLC analysis, and no timely quantitation is necessary. In addition, this method offers some potential to detect adulteration with other contaminants, especially those that have high ratios of either γ- or δ-tocopherol, since coffee has a lower ratio of both. Tocopherol fingerprinting could be used by researchers to survey a large number of coffee samples and should be investigated to detect adulteration of coffee by other grains and cereals, that is, soybeans also supposedly used for adulteration. One of the problems was the low concentration of γ-tocopherol in coffee and limited sensitivity of fluorescence detection. Other more sensitive methods, such as chromatography coupled with mass spectrometry, should be evaluated. In addition, other markers such as fatty acids, sterols, triacylglycerols, organic acids, etc. should also be investigated.

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