JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Natural Occurrence of Ochratoxin A and Antioxidant Activities of Green and Roasted Coffees and Corresponding Byproducts

AURORA NAPOLITANO, VINCENZO FOGLIANO, ALESSIO TAFURI, AND ALBERTO RITIENI*

Dipartimento di Scienza degli Alimenti, Università di Napoli "Federico II", Via Università 100, Parco Gussone, 80055 Portici, Napoli, Italy

Ochratoxin A is an important mycotoxin that can enter the human food chain in cereals, wine, coffee, spices, beer, cocoa, dried fruits, and pork meats. Coffee is one of the most common beverages and, consequently, it has a potential risk factor for human health related to ochratoxin A exposure. In this study, coffee and corresponding byproducts from seven different geographic regions were investigated for ochratoxin A natural occurrence by HPLC-FLD, nutritional characterization, and antioxidant activities by spectrophotometric assay. The research focused on composition changes in coffee during the processing step "from field to cup". Costa Rica and Indian green coffees were the most contaminated samples, with 13 and 11 μg/kg, respectively, while the Ethiopian coffee was the least contaminated, with 3.8 μg/kg of ochratoxin A. The reduction of ochratoxin A contamination during the roasting step was comparable for any samples that were considered under the recommended level of 4 μg/kg. Total dietary fibers ranged from 58.7% for Vietnam and 48.6% for Ivory Coast in green coffees and ranged from 58.6% for Costa Rica to 61.2% for India in roasted coffee. Coffee silverskin byproduct obtained from Ivory Coast was the highest, with 69.2 and 64.2% of insoluble dietary fibers, respectively.

KEYWORDS: Ochratoxin A; coffee; dietary fiber; silverskin; antioxidant activity

INTRODUCTION

Coffee beans, green coffee, roasted coffee, and the beverages prepared from roasted beans are very complex mixtures of several hundreds of compounds that are both naturally occurring and induced by roasting process. The chemistry and the biological activities of these substances have not yet been completely elucidated.

The most important botanical coffee species and varieties are *Coffea arabica* and *Coffea robusta*, which account, respectively, for about 75 and 24% of the world production. Coffee can be cultivated only in subtropical, tropical, or equatorial climatic conditions and Arabica coffee is a highland species, adapted to high altitudes where lower temperatures and reduced humidity prevail. In contrast, Robusta is a lowland species cultivated from sea level up to 1000 m (*1*). These two species exhibit considerable differences in their botanical, genetic, agronomical, chemical, and morphological characteristics. The characteristic flavor of Robusta is less influenced by climatic conditions than is that of Arabica; both species' flavor development is the result of pyrolytic reactions that occur during roasting processes (*1*).

The chemistry and the composition of coffee beverages are strictly related to the two methods employed in green coffee processing, the dry and wet methods. However, both have peculiarities dependent on the geographical regions, because the production procedures are not optimized, even in the same country. In the dry method, which is very common in Brazil, coffee fruit is dried in its whole (cherry) state, together with pulp and mucilage. The wet method is more sophisticated and requires the elimination of pulp and mucilage before coffee fruit drying (parchment state). The quality of the wet product is higher than that of the dry product, and the corresponding roasted coffees have been associated with a better sensory quality; the latter method is employed when a smooth Arabica coffee is sought. The fermentation step occurs in the water, allowing lower temperatures, which is probably the main reason for improved quality. In addition, the roasting step of green coffee is chemically a complex process because hundreds of chemical reactions take place simultaneously in the coffee beans. Some important examples of these transformations include Maillard and Strecker reactions, as well as the degradation of proteins, polysaccharides, trigonelline, and chlorogenic acids. Because of the greater fraction of cellulose and the introduction of hydrolysis byproduct acids and aromatics, both types of instant coffee are very different in composition from ground-roasted coffee or the soluble product obtained from brewed coffee.

The tegument of coffee beans (outer layer), coffee silverskin, is the main byproduct of the roasting procedures.

* Corresponding author (e-mail alberto.ritieni@unina.it; telephone + 39 0812539351; fax + 39 0817754942).

10.1021/jf071959+ CCC: $37.00
Published on Web 11/20/2007

Coffee silverskin is used in Western countries as a soil fertilizer or combustible (*2*), but this byproduct importance is due to the attested health properties in coffee brews. Thus, it is conceivable that some properties, such as antioxidant activity or anticarcinogenic effects, are also retained in the tegument. Borrelli and co-workers (*3*) reported the composition of coffee silverskin, obtained from several Italian roasting plants, showing a high amount of soluble dietary fiber (about 14% of the total fiber) and a very high antioxidant activity. The phenolic acids contents in beverages varied widely, ranging from 0 (pear cider) to 97 mg/100 g (special coffee). In most beverages, the hydrolysis procedures produce a large amount of phenolic acids from their bound forms. Coffee and black teas are exceptions; the aglycone levels of phenolic acid were comparable after thermal treatments (*4*). However, most studies on coffee associate negative aspects to toxicity of caffeine to human health (*5, 6*).

Coffee is a tropical product and most likely to be colonized by *Aspergillus* species, typified by *A. ochraceus* and *A. carbonarius*, synthesizing mycotoxins such as ochratoxin A (*7*).

Ochratoxin A natural occurrence in green and roasted coffee is widely described in the literature (*8*). In particular, Romani et al. (*9*) showed that 106 of 162 samples of green coffee beans from various countries were positive for ochratoxin A, with concentrations up to 48 ppb. Ochratoxin A can occur in coffee beans in several environmental conditions (climatic conditions, abnormally long storage, and transportation) and processing conditions (wet or dry process) (*10, 11*). It might be possible to reduce ochratoxin A contamination of green coffee by understanding the stage and conditions under which this compound is produced during green coffee production and handling (*12*). Ochratoxin A is already present before storage; the critical steps to accumulate this mycotoxin are the harvesting and the postharvest handling of coffee cherries. A partial ochratoxin A degradation occurs during the roasting process, reaching levels of 69% reduction (*13*). To ensure the healthiness and the sensory quality of the coffee, adoption of a preventive quality control system based on identifying and controlling the critical points along the processing chain has been proposed (*14*).

The objectives of the present investigation are the chemical characterization of green coffee beans, coffee silverskin, and roasted coffee and the corresponding coffee beverages and, critically, the estimation of the natural occurrence of ochratoxin A in the same samples corresponding to whole coffee chain from field to cup.

MATERIALS AND METHODS

Sampling. Green and roasted coffee samples as well as corresponding coffee silverskin were kindly provided by Caffè Moak Ltd. (Italy) from different geographic locations. Each sample was of 5 kg, and each one represents a coffee bulk of five. Three *Coffea robusta* samples were from Ivory Coast (IC), Vietnam (VN), and Cameroon (CM), whereas four *Coffea arabica* samples were from Ethiopia (ET), Santos (S), India (I), and Costa Rica (CR). A morphological examination of green coffee supplied confirmed the botanical classification and geographical origin. A standard industrial and unique method for any sample was used to roast the coffee to eliminate variations due to this process. All samples were hermetically sealed and stored under vacuum at −20 °C and in the dark until chemical analysis. Roasted coffee beans were ground to an appropriate particle size for the espresso brewing technique by using a professional grinder. All samples were collected at the end of the drying process and had a moisture level in the range between 11 and 13%. Espresso coffee beverages were prepared by using a household espresso coffee machine (Gaggia, Italy) under the following conditions: coffee powder, 13.0 g; extract (beverage) mass, 50 g according to standard espresso coffee preparation techniques (*15*).

Reagents. *N,N*-Dimethyl-*p*-phenylenediamine and 2,2-azinobis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) were obtained from Fluka (Buchs, Switzerland). An enzymatic kit to determine fiber content was from Megazyme; it contained an R-amylase (E-BLAAM) with an activity of 3.000 Ceralpha units/mL, a protease (E-BSPRT) at a concentration of 50 mg/mL (350 tyrosine units/mL), and an amyloglucosidase (E-AMGDF) with an activity of 200 *p*-nitrophenol-α-maltoside units/mL. Ochratoxin A standard was purchased from Sigma-Aldrich (St. Louis, MO). Other chemicals were from Sigma-Aldrich. All solvents used were of HPLC-grade and obtained from Merck (Darmstadt, Germany).

Apparatus. Enzymatic incubation was performed in a covered water bath with shaking (Stuart Scientific). A UV 2100 spectrometric apparatus (Shimadzu, Kyoto, Japan) was used to evaluate antioxidant capacity. The amount of fat was determined in a Soxhlet apparatus (Kimble Kontes, Vineland, NJ).

Experimental Procedures. *Chemical Composition.* Chemical composition of all coffee samples was determined following standard methods (*16*). In particular, total sugar percentage was determined after acid hydrolysis, as reported by Selvendran (*17*); reducing sugars were evaluated by using the Fehling method and total proteins bu following the Kjeldahl method. Total fat amounts were determined with a Soxhlet apparatus; and moisture and dry weight were determined by gravimetric methods. Caffeine concentration for each sample was determined as described by the AOAC (*18*). The experiments were replicated three times for each sample.

Phosphate Buffer Solution (PBS). The phosphate buffer solution was prepared from potassium chloride (0.2 g), potassium dihydrogen/phosphate (0.2 g), anhydrous disodium hydrogen/phosphate (1.2 g), and sodium chloride (8 g) added to distilled water (900 mL). After dissolution, the pH was adjusted to 7.4 with 0.1 mol/L HCl or 0.1 mol/L NaOH as appropriate.

Antioxidant Activities. Each sample was evaluated by measuring aqueous and methanol extract antioxidant activities representing hydrophilic and lipophilic molecules. The extractions were performed as follows: to 1 g of each sample was added 5 mL of distilled water or pure methanol, and the samples were then spun in a refrigerated centrifuge at 4000 rpm for 5 min. This procedure was performed twice. The supernatants were removed and filtered (0.45 μm) before being stored in the dark at 4 °C until analysis. The water-soluble antioxidant activity of aqueous extracts was measured according to the *N,N*-dimethyl-*p*-phenylenediamine method (*19*), whereas the lipophilic antioxidant activity of methanol extracts was evaluated by using the 2,2′-azinobis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) method as described (*20*).

Determination of Soluble and Insoluble Fiber. The amounts of soluble dietary fiber (SDF) and insoluble dietary fiber (IDF) were determined according to a gravimetric enzymatic method that requires two steps: enzymatic digestion and filtration (*21*).

Enzymatic Digestion. Samples (1 g) were suspended in 25 mL of Tris-Mes buffer (0.05 mM), pH 8.2, and incubated at 100 °C for 35 min with 50 μL of di-α-amylase. After cooling to 60 °C, 100 μL of the protease mixture was added to the solution, which was then incubated at 60 °C. Thirty minutes later, the reaction was stopped by adding 5 mL of 0.56 M HCl with a pH value between 4.1 and 4.8. Finally, 200 μL of amyloglucosidase was added, and the solution was incubated at 60 °C for 30 min.

Filtration and Determination of IDF and SDF. IDF was obtained by filtering the ethanol solution through a crucible with a Celite filter. Subsequently, insoluble material was repeatedly washed as follows: three washings with 25 mL of ethanol (78%); two washings with 10 mL of ethanol (90%); and two washings with 10 mL of acetone. The crucible was placed at 105 °C overnight and then weighed. It was placed

Table 1. Coffee Silverskin Nutritional Composition (Grams per 100 g of Product)[a]

component	Cameroon	Ivory Coast	Costa Rica	Ethiopia	Indian	Santos	Vietnam
proteins	18.60 ± 0.3 a	19.0 ± 0.5 a	18.4 ± 0.1 a	18.5 ± 0.4 a	19.0 ± 0.3 a	19.0 ± 0.5 a	17.9 ± 0.4 a
fats	2.50 ± 0.1 a	2.92 ± 0.5 a	1.56 ± 0.1 b	3.15 ± 0.2 a	2.20 ± 0.1 a	3.28 ± 0.3 a	2.60 ± 0.1 a
carbohydrates	80.50 ± 1.2 b	47.0 ± 1.6 a	47.0 ± 1.2 a	34.6 ± 1.3 a	52.0 ± 1.3 a	34.6 ± 1.3 a	52.0 ± 1.6 a
reducing sugar	nd	nd	nd	nd	nd	nd	nd
caffeine	1.35 ± 0.2 b	0.81 ± 0.1 a	1.16 ± 0.05 a	1.16 ± 0.03 a	0.83 ± 0.05 a	0.97 ± 0.1 a	1.37 ± 0.3 b
TDF	53.40 ± 0.3 a	69.2 ± 0.3 b	62.4 ± 0.3 a	65.9 ± 0.5 a	57.9 ± 0.6 a	56.4 ± 0.3 a	67.7 ± 0.3 b
IDF	48.50 ± 0.4 a	64.2 ± 0.5 b	57.4 ± 0.5 b	60.7 ± 0.3 b	52.7 ± 0.2 a	50.1 ± 0.1 a	58.4 ± 0.2 b
SDF	4.90 ± 0.6 a	5.0 ± 0.2 a	5.0 ± 0.5 a	5.2 ± 0.3 a	5.2 ± 0.1 a	6.3 ± 0.5 a	9.3 ± 0.1 b

[a] Each value is a mean of triplicate analyses ± SD. Mean values with the same letter in the same row do not differ significantly.

for 5 h in a muffle furnace at 505 °C and then weighed. The percentage of IDF was calculated as follows:

$$\text{IDF}\,\% = \frac{\{R - [(p + A/100) \times R]\} - B}{M \times 100}$$

All values are expressed in grams, where M = sample weight, R = total solids from M, p = protein weight, A = ash weight from R, and B = weight of blank (enzymes + solvents without sample)

$$B = \text{BR} - \text{BP} - \text{BA}$$

where BR = total solids from B, BA = ashes from BR, and BP = protein from BR.

SDF was obtained by adding 300 mL of ethanol at 60 °C to the filtered fractions collected from IDF separation after 1 h. The SDF was precipitated and recovered after 1 h by filtration of the solution on a crucible with a Celite filter. Subsequently, insoluble material was repeatedly washed as follows: three washings with 25 mL of ethanol (78%); two washings with 10 mL of ethanol (90%); and two washings with 10 mL of acetone. The crucible was placed at 105 °C overnight and then weighed. Then, it was placed for 5 h in a muffle furnace at 505 °C and again weighed. SDF was calculated from the same formula used for IDF evaluation. All samples were analyzed in triplicate for statistical analyses.

Extraction of Ochratoxin A and Cleanup Procedures. Each sample was analyzed for ochratoxin A according to the high-performance liquid chromatography (HPLC) method as described by Solfrizzo et al. (*22*). In particular, 100 mL of a solution of acetonitrile (40%) was added to 25 g of ground coffee sample and then was shaken vigorously for 60 min to homogenize the solution. The suspension was separated by centrifugation at 4000 rpm for 10 min, and 5 mL of supernatant was added to 55 mL of PBS (0.2 M, pH 7.2) solution. The clean-up of the samples was performed using an Ochratest specific immunoaffinity column (IAC) (Vicam); 12 mL of supernatant diluted solution was passed through the IAC at a maximum rate of 1–2 drops/s. To clean the IAC column, 10 mL of PBS solution followed by 10 mL of distilled water were passed at the same rate. Finally, ochratoxin A was eluted from column with 1 mL of methanol with collection in a Pyrex glass test tube. The residue was resuspended in 1 mL of HPLC mobile phase and filtered through a 0.20 μm RC025 disk filter (Phenomenex). Detection and quantification were performed by HPLC using a chromatograph system equipped with an RF-10A_{XL} fluorescent detector, a system controller of two binary pumps model LC-10AD_{VP} (Shimadzu), and a 150 × 4.6 mm C_{18} HL 90-5 S Column (Bio-Rad). A binary chromatographic time program (0.0 min, 50% B; min, 15.0 min, 100% B; 15.0 min; 18.0 min, 50% B; 23.0 min, 50% B) was used with a mobile phase composed of acetonitrile/acetic acid (49:1) (B) and water/acetic acid (49:1) (A) pumped at a constant flow rate of 1 mL/min. The fluoriometric excitation and emission wavelengths were set at 333 and 460 nm, respectively. Ochratoxin A was quantified by comparison with external standards (Sigma, purity > 98%). The ochratoxin A detection limit was 1 ng/kg, and the quantification limit was 0.1 μg/kg. Mean recoveries from spiked samples (n = 3) at levels of 5.0–50 μg of ochratoxin A/kg were 72% (RSD = 2.9%) for coffee powder (coffee silverskin, green coffee, and roasted coffee) and 92% (RSD = 2.1%) for coffee beverage.

LC/MS/MS Analyses. Samples were analyzed in triplicate and, when showing ochratoxin A concentrations of >100 ng/kg, were analyzed by HLPC-MS-MS API 3000 equipped with Turbo Ion Spray Source (Applied Biosystem, Ontario, Canada) for structural confirmation. All analyses were performed using drying gas (N_2) at 400 °C.

Chromatographic separations were performed on a Gemini 5u C_{18} 110A, 150 × 2 mm (Phenomenex), column using the following mobile phases, water/0.2% formic acid (solvent A) and methanol/acetonitrile (90:10 v/v) (solvent B). The following gradient elution was applied: 0–12 min, 80% B; 12–13 min, 100% B; 13–18 min, 100% B. The quantification was carried out in multiple reaction monitoring (MRM). The limit of detection was 10 ng/kg (signal/noise ratio = 3), and the limit of quantification was 100 ng/kg (signal/noise ratio = 6). These experiments were simultaneously performed and replicated four times.

Statistical Analysis. Statistical analysis of data was performed on the original data by analysis of variance; significance was assessed by using the Tukey test, which allowed a multiple comparison among the data to individualize the significant differences. Differences were considered significant if $p < 0.05$. All values are presented ± SD.

RESULTS AND DISCUSSION

Recent epidemiological studies have established an association between the common consumption of coffee, or other caffeinated beverages, and a reduced risk of developing neurodegenerative diseases, such as Parkinson's disease (*23*). It has also been reported that, at low doses, coffee can suppress the in vitro mutagenicity of oxidants such as *tert*-butyl hydroperoxide. Coffee can also inhibit lipid peroxidation and malondialdehyde synthesis. Furthermore, roasted coffee had strong antiperoxyl radical activity in a chemical system (linoleic acid–α-carotene micellar system) and in a biological system (rat liver microsomes) (*24*).

Composition. Literature data are available on the composition of coffee (green beans, roasted beans, or prepared) focusing on food quality aspects or adulteration with non coffee products (*25*).

Table 1 summarizes the chemical composition of coffee silverskin, whereas **Table 2** describes the parameters for green and roasted samples, respectively. **Table 3** explains the most important coffee beverage characteristic. Carbohydrates occur in green coffee, as both soluble and insoluble polysaccharides, at levels from 49 to 88%. The insoluble cell walls, which are identical in composition in both Arabica and Robusta, are composed of cellulose and hemicellulose, and are about 3% higher in Robusta according to literature data (*26*). In roasted samples, the values range from 59.7% (in Vietnam coffee) to 71.2% (in Costa Rica coffee). On the other hand, polysaccharides, mainly arabinogalactans and galactomannans, represent the main components of coffee silverskin, with values from 34 to 80%, in particular mainly arabinogalactans and galactomannans, in accord with Nunes and Coimbra (*27*). Reducing carbohydrates are almost absent. Fat concentration is very low in green coffee and ranges between 9.7 and 15.4% in roasted coffee and, particularly, was the marked amount of protein.

Table 2. Green Coffee (Italic Typeface) and Roasted Coffee (Roman Typeface) Sample Nutritional Composition (Grams per 100 g of Product)[a]

component	Cameroon	Ivory Coast	Costa Rica	Ethiopia	Indian	Santos	Vietnam
proteins	*16.4 ± 0.13 a*	*14.2 ± 0.15 a*	*11.7 ± 0.18 a*	*18.8 ± 0.15 b*	*18.8 ± 0.13 b*	*20.3 ± 0.8 b*	*12.5 ± 0.12 a*
	12.1 ± 0.13 a	15.7 ± 0.18 a	13.4 ± 0.15 a	12.2 ± 0.17 a	11.6 ± 0.12 a	11.0 ± 0.13 a	12.5 ± 0.14 a
fats	*6.2 ± 0.5 b*	*7.4 ± 0.2 a*	*8.7 ± 1.0 a*	*8.8 ± 0.2 a*	*8.6 ± 0.3 a*	*7.8 ± 1.0 a*	*5.7 ± 0.7 a*
	14.7 ± 0.2 a	9.7 ± 0.3 b	13.1 ± 0.4 a	15.4 ± 0.3 a	10.2 ± 0.5 b	15.4 ± 0.3 a	9.74 ± 0.1 b
carbohydrates	*52.6 ± 1.2 a*	*75.7 ± 1.5 b*	*66.0 ± 1.2 b*	*49.1 ± 1.5 a*	*75.7 ± 1.0 b*	*58.5 ± 1.2 a*	*88.8 ± 0.2 b*
	70.0 ± 1.3 a	69.8 ± 1.3 a	71.2 ± 1.5 a	69.8 ± 1.8 a	69.8 ± 1.5 a	69.8 ± 1.6 a	59.7 ± 1.0 a
reducing sugar	*nd*	*nd*	*nd*	*nd*	*n.d*	*nd*	*nd*
	nd	nd	nd	n.d	nd	nd	nd
moisture	*10.2 ± 0.2 a*	*8.5 ± 0.1 a*	*9.5 ± 0.2 a*	*9.6 ± 0.3 a*	*7.1 ± 0.3 a*	*9.6 ± 0.1 a*	*2.1 ± 0.2 b*
	2.1 ± 0.1 a	2.9 ± 0.3 a	2.5 ± 0.1 a	2.0 ± 0.1 a	2.2 ± 0.2 a	2.5 ± 0.3 a	2.1 ± 0.1 a
ashes	*4.6 ± 0.1 a*	*4.2 ± 0.5 a*	*4.1 ± 0.3 a*	*3.6 ± 0.6 a*	*3.5 ± 0.2 a*	*3.8 ± 0.3 a*	*1.2 ± 0.2 b*
	1.1 ± 0.13 a	1.9 ± 0.13 a	1.8 ± 0.13 a	1.8 ± 0.13 a	1.6 ± 0.13 a	1.2 ± 0.13 a	1.0 ± 0.13 a
caffeine	*1.6 ± 0.5 a*	*1.2 ± 0.3 a*	*0.5 ± 0.5 b*	*1.2 ± 0.6 a*	*1.6 ± 0.5 a*	*1.2 ± 0.3 a*	*0.9 ± 0.3 b*
	0.6 ± 0.1 a	0.6 ± 0.2 a	0.4 ± 0.3 a	0.5 ± 0.5 a	0.5 ± 0.4 a	0.4 ± 0.2 a	0.7 ± 0.3 a
TDF	*50.0 ± 0.2 a*	*48.6 ± 0.3 a*	*49.2 ± 0.5 a*	*51.3 ± 0.5 a*	*49.5 ± 0.2 a*	*51.0 ± 0.3 a*	*58.7 ± 0.2 b*
	59.7 ± 0.5 a	60.2 ± 0.8 a	58.6 ± 0.8 a	59.3 ± 0.7 a	61.2 ± 0.9 a	60.3 ± 0.5 a	59.7 ± 0.3 a

[a] Each value is a mean of triplicate analysis ± SD. Mean values with the same letter in the same row do not differ significantly.

Table 3. Coffee Beverage Composition (Grams per 100 mL)[a]

component	Cameroon	Ivory Coast	Costa Rica	Ethiopia	Indian	Santos	Vietnam
proteins	0.2 a	0.5 a	0.3 a	0.4 a	0.4 a	0.2 a	0.3 a
total lipids	2.1 b	0.8 a	0.6 a	0.7 a	0.9 a	1.0 b	2.5 b
soluble carbohydrates	8.0 a	9.6 a	9.3 a	8.6 a	9.2 a	9.6 a	7.5 a
caffeine (mg)	59.0 a	60.0 a	48.0 b	50.0 b	48.0 b	58.0 a	60.0 a
ashes	7.0 a	7.1 a	7.5 a	7.6 a	6.8 a	6.8 a	7.5 a

[a] Mean values with the same letter in the same row do not differ significantly.

There is no evidence to suggest that the protein content of coffees of different qualities or even of different species (Arabica vs Robusta) should be noticeably different (*28*). Protein levels of green coffee were also in the range reported in the literature: 11–16.5 g/100 g (*28*). It is noteworthy that this result was based on the determination of crude nitrogen and multiplication by a factor of 6.25.

The ash value is high due to mineral content, and after roasting, the moisture levels decreased to an average of 1.5 g/100 g, with no differences among the samples. Within the framework of this study, it appears that the geographic origin of coffees may determine their chemical profiles. However, the average content of caffeine is 1.2% in Arabica and 2.2% in Robusta, showing a marked interspecific difference as well as high intraspecific variability; there was a 30% reduction of caffeine content after the roasting step due to organic losses (*29*).

Soluble fiber, insoluble fiber, and total dietary fiber values are summarized in **Table 1**, and the highest value of TDF as described by Borrelli (*4*) was in the Ivory Coast coffee; TDF values varied significantly ($p < 0.05$) and ranged from 53.4% for Cameroon to 69.2% for Ivory Coast. IDF levels ranged from 48.5% for Cameroon to 64.1% for Ivory Coast, indicating IDF to be a major component of the fiber material in the silverskin byproduct of the roasting process.

All composition data obtained were in accord with the literature and suggest that no significant correlations occurred between geographic variety, growth conditions, and chemical composition.

Antioxidant Activity. Polyphenols are an important source of dietary antioxidants, being distributed widely in fruits, vegetables, cereals, and beverages, including red wine, tea, coffee, and cocoa. Roasting markedly affects the composition of the coffee polyphenols obtained through the Maillard reaction and confers to coffee its pleasant taste and aroma (*30*). In addition, carbohydrate caramelization and the pyrolysis of organic compounds occur during the process. However, although natural antioxidants were lost during heating, the overall antioxidant properties of coffee brews can be maintained or enhanced by the formation of new antioxidants such as the Maillard reaction products. The concentration of the Maillard products in the total solid matter of roasted coffee is about 20%, with the highest levels of these compounds present in medium-roasted samples, and these results agree with those of Nicoli et al. (*31*).

It can be hypothesized that the antioxidant activity was intimately related to the intensity of the thermal treatment employed in the roasting of the coffee beans. Medium-roasting conditions could be the most appropriate to obtain optimal antioxidant properties. However, as a consequence of the complexity of this matrix and the reactions that can take place during coffee roasting, more studies are necessary to clarify the identity and antioxidant mechanism of the different compounds responsible for these properties. The trends in the amounts of antioxidants molecules were comparable for Arabica and Robusta coffees. In green coffee bean samples, the antioxidant content ranged from 0.91 mmol of Trolox/100 g for Ivory Coast to 0.71 mmol of Trolox/100 g for Santos and increased for all samples after the roasting process, as shown in **Figure 1**. The increase was not linear, as Ivory Coast, which started with the highest quantity of antioxidant, was characterized after the thermal process as possessing the lowest amount of antioxidant compounds (1.2 mmol of Trolox/100 g). High levels of

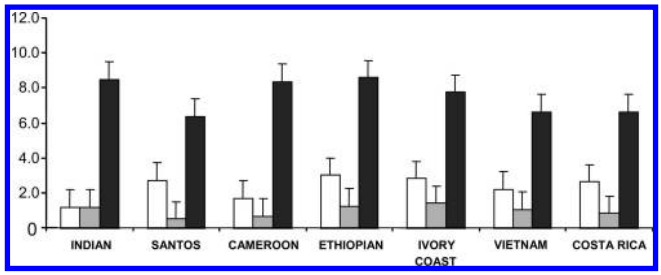

Figure 1. Antioxidant activity of coffee samples expressed as millimoles of Trolox per 100 g of product ($ABTS^{+}$): (white bars) coffee silverskin; (gray bars) green coffee; (black bars) roasted coffee ($p < 0.05$).

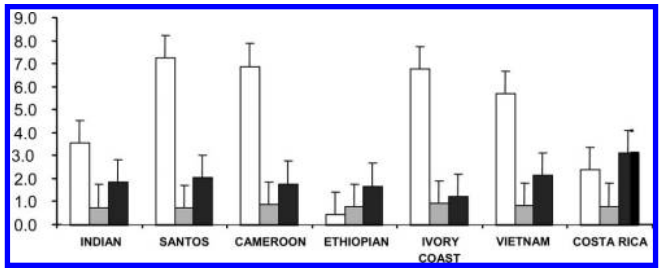

Figure 2. Antioxidant activity of coffee samples expressed as millimoles of ascorbic acid per 100 g of product: (white bars) coffee silverskin; (gray bars) green coffee; (black bars) roasted coffee. ($p < 0.05$).

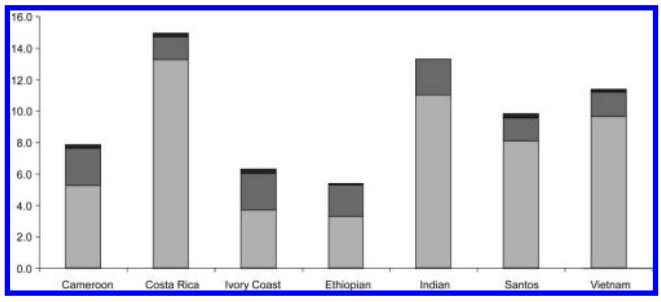

Figure 3. Ochratixin A content of coffee samples expressed as parts per billion: (light gray) green coffee; (dark gray) roasted coffee; (black) coffee beverage ($p < 0.05$).

antioxidant activities were reported for coffee silverskin (*4*); in the coffee silverskin samples of this study, antioxidant activity was of 2.08 mmol of Trolox/100 g, with the highest value for the Ethiopian sample, 8.2 mmol of Trolox/100 g. Coffee silverskin methanol extracts have an antioxidant activity comparable to that of wheat bran, which is known to have a very high antioxidant activity of 1.9 mmol of Trolox/100 g. The antioxidant activity of coffee silverskin is related to part of the polyphenolic compounds that are normal constituents of coffee beans.

The *N*,*N*-dimethyl-*p*-phenylenediamine method was applied to evaluate the activity of water-soluble compounds; in coffee silverskin, the antioxidant compounds extracted were the most abundant, with the highest value for Santos samples (7 mmol of ascorbic acid/100 g). After the roasting process, a dramatic increase was observed for all samples analyzed, with a median difference of 53% for green and roasted coffee (**Figure 2**).

Ochratoxin A Reduction. Figure 3 shows that ochratoxin A absolute quantities in the residues after roasting are always reduced for all samples. The hypothesis is that thermal destruction and chaff removal are two process steps involved in the reduction of ochratoxin A occurrence from powder to cup. The reduction performances are strongly influenced by the severity of the thermal process used and initial ochratoxin A content in the raw material (*32*). As show in **Table 4**, after thermal

Table 4. Ochratoxin A Behavior in Coffee Beverage Preparation

coffee	roasted coffee (ng)	coffee beverage (ng)	extracted mycotoxin (%)
Indian	29.9	15.0	50
Santos	18.2	15.0	82
Cameroon	29.9	10.0	33
Ethiopia	26.0	5.0	19
Ivory Coast	29.9	15.0	50
Vietnam	19.5	10.0	51
Costa Rica	19.5	10.0	50

Roasted coffee, ng in 13 g of coffee powder; coffee beverage, ng in 50 mL of beverage.

treatment, the ochratoxin A occurrences are comparable for each sample, with the values ranging between 1.4 and 2.3 μg/kg. Several studies mention physical removal of ochratoxin A with the coffee silverskin (chaff) as a possible mechanism to clarify the reduction, but the literature data are only a partial explanation of ochratoxin A removal (*33*). The processing conditions adopted to obtain samples suitable for a typical espresso coffee brew showed a reduction of the ochratoxin A content in samples with both high and low contamination levels. For a better understanding of the data, ochratoxin A levels of coffee beverage samples are reported as nanograms of ochratoxin A content initially occurring in 50 mL of beverage obtained from 13 g of coffee powder (roasted coffee) (**Table 4**). The natural occurrence of ochratoxin A in roasted coffee is reduced during soluble coffee manufacture; in fact, mycotoxin was detected in the beverage in reduced quantities ranging from 19% in Ethiopian sample to 82% in Santos coffee of initial amount, with respect to attended values of about 17% of the initial powder composition. The residual ~12% ochratoxin A present in the final beverage indicates a further reduction during soluble coffee manufacture, and a cup of coffee prepared from 2 g of powder would contain ~2 ppb of ochratoxin A.

The most important results of this study indicate that both roasted and ground coffee and soluble coffee are secondary sources of ochratoxin A in the human diet, even when prepared from relatively highly contaminated green beans. Ochratoxin A intake through coffee beverage cannot be considered a primary source, and the coffee-transforming process is able to reduce this critical point and related risk for human health.

Finally, because many samples are available from different locations, it is possible to hypothesize that there are no significant differences in ochratoxin A occurrence between the two main varieties Arabica and Robusta in the samples evaluated in this study.

ABBREVIATIONS USED

DF, dietary fiber; SDF, soluble dietary fiber; IDF, insoluble dietary fiber; CS, coffee silverskin; OTA, ochratoxin A.

ACKNOWLEDGMENT

We are indebted to Progeotech Enterprise and to Eng. Domenico Palmieri for technical support.

LITERATURE CITED

(1) de Carvalho, V. D.; de Chagas, S. J. R.; de Souza, S. M. C. Factores que afetam a qualidade do càfe. *Inf. Agropecuàrio, Belo Horizonte* **1997**, *18* (187), 5–20.

(2) Saenger, M.; Hartge, E. U.; Werther, J.; Ogada, T.; Siagi, Z. Combustion of coffee husks. *Renewable Energy* **2001**, *23*, 103–121.

(3) 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.
(4) Mattila, P.; Hellstro, J.; Torronen, R. Phenolic acids in berries, fruits, and beverages. *J. Agric. Food Chem.* **2006**, *54*, 7193–7199.
(5) Carrillo, J. A.; Benitez, J. CYP1A2 activity, gender and smoking, as variables influencing the toxicity of caffeine. *Br. J. Clin. Pharmacol.* **1996**, *41*, 605–608.
(6) Derlet, R. W.; Tseng, J. C.; Albertson, T. E. Potentiation of cocaine and α-amphetamine toxicity with caffeine. *Am. J. Emerg. Med.* **1992**, *10*, 211–216.
(7) Mantle, P. G.; Chow, A. M. Ochratoxin formation in *Aspergillus ochraceus* with particular reference to spoilage of coffee. *Int. J. Food Microbiol.* **2000**, *56*, 105–109.
(8) Otteneder, H.; Majerus, P. Ochratoxin A (OTA) in coffee: nationwide evaluation of data collected by German Food Control 1995–1999. *Food Addit. Contam.* **2001**, *18*, 431–435.
(9) Romani, S.; Sacchetti, G.; Chaves Lopez, C.; Pinnavaia, G. G.; Dalla Rosa, M. Screening on the occurrence of ochratoxin A in green coffee beans of different origins and types. *J. Agric. Food Chem.* **2000**, *48*, 3616–3619.
(10) Smith, J. E.; Solomons, G. L.; Lewis, C. W.; Anderson, J. G. *Mycotoxins in Human Nutrition and Health*; Technical Report; European Commission, Directorate XII: Studies on Science, Research and Development (SRD); EUR 16048 EN; SRD: Brussels, Belgium, 1994.
(11) Viani, R. Fate of ochratoxin A (OTA) during processing of coffee. *Food Addit. Contam.* **1996**, *13*, 29–33.
(12) Bucheli, P.; Meyer, I.; Pittet, A.; Vuataz, G.; Viani, R. Industrial storage of green Robusta coffee under tropical conditions and its impact on raw material quality and ochratoxin A content. *J. Agric. Food Chem.* **1998**, *46*, 4507–4511.
(13) Van der Stegen, G. H.; Essens, P. J.; Van der Lijn, J. Effect of roasting conditions on reduction of ochratoxin A in coffee. *J. Agric. Food Chem.* **2001**, *49*, 4713–47.
(14) FAO.X *Preventing Mycotoxin Contamination*; Food Nutrition and Agriculture 23; FAO: Rome, Italy, 1999.
(15) Petracco, M. Beverage preparation: brewing trends for the new millennium. In *Coffee Recent Developments*; Clarke, R. J., Vitzthum, O. G., Eds.; Blackwell Science: Oxford, U.K., 2001; 140.
(16) Association of Official Analytical Chemists. *Official Methods of Analysis of AOAC International*, 16th ed.; AOAC: Gaithersburg, MD, 1995.
(17) Selvendran, R. R.; Stevens, B. J.; Du Pont, M. S. Dietary fiber: chemistry, analysis, and properties. *Adv. Food Res.* **1987**, *31*, 117–209.
(18) Association of Official Analytical Chemists. *Official Methods of Analysis of AOAC International*; AOAC: Gaithersburg, MD, 1975.
(19) Fogliano, V.; Verde, V.; Randazzo, G.; Ritieni, A. Method for measuring antioxidant activity and its application to monitoring the antioxidant capacity of wines. *J. Agric. Food Chem.* **1999**, *47*, 1035–1040.
(20) Pellegrini, N.; Yang, M.; Rice-Evans, C. Screening of dietary carotenoids and carotenoids-rich frits extracts for the antioxidant activities applying ABTS radical cation decolorization assay. *Methods Enzymol.* **1999**, *299*, 379–389.
(21) Prosky, L.; Asp, N. G.; Schweizer, T. F.; DeVries, J. W.; Furda, I. Determination of unsoluble, soluble, and total dietary bran foods and food products. Interlaboratory study. *J. AOAC Int.* **1998**, *71*, 1017–1023.
(22) Solfrizzo, M.; Avantaggiato, G.; Visconti, A. Use of various cleanup procedures for the analysis of ochratoxin A in cereals. *J. Chromatogr., A* **1998**, *815*, 67–73.
(23) Chen, F.; Xu, K.; Petzer, J. P.; Staal, R.; Xu, Y. H.; Beilstein, M.; Sonsalla, P. K.; Castagnoli, K.; Castagnoli, N., Jr.; Schwarzschild, M. A. Neuroprotection by caffeine and A2A adenosine receptor inactivation in a model of Parkinson's disease. *J. Neurosci.* **2001**, *21*, 1–6.
(24) Daglia, M.; Papetti, A.; Gregotti, C.; Berte, F.; Gazzani, G. In vitro antioxidant and ex vivo protective activity of green and roasted coffee. *J. Agric. Food Chem.* **2000**, *48*, 1449–1454.
(25) Yeretzian, C; Jordan, A.; Badoud, R.; Lindinger, W. From the green bean to the cup of coffee: investigating coffee roasting by on-line monitoring of volatiles. *Eur. Food Res. Technol.* **2004**, 92–104.
(26) Bradbury, A. G. W.; Halliday, D. J. Chemical structure of green coffee bean polysaccharides. *J. Agric. Food Chem.* **1990**, *38*, 389–392.
(27) Nunes, F. M.; Coimbra, M. A. Chemical characterization of the high-molecular-weight material extracted with hot water from green and roasted robusta coffees as affected by the degree of roast. *J. Agric. Food Chem.* **2002**, *50*, 7046–7052.
(28) Macrae, R. In *Nitrogenous Compounds*; Clarke, R. J., Macrae, R., Eds.; Elsevier Applied Science: London, U.K., 1985; Vol. 1, pp 115–152.
(29) Franca, A. S.; Mendonça, J. C. F.; Oliveira, S. D. Composition of green and roasted coffees of different cup qualities. *Food Sci. Technol. Res./LWT* **2005**, *7*, 709–715.
(30) Richelle, M.; Tavazzi, I.; Offord, E. Comparison of the antioxidant activity of commonly consumed polyphenolic beverages (coffee, cocoa, and tea) prepared per cup servine. *J. Agric. Food Chem.* **2001**, *49*, 3438–3442.
(31) Nicoli, M. C.; Anese, M.; Parpinel, M. T.; Franceschi, S.; Lerici, C. R. Loss and/or formation of antioxidants during food processing and storage. *Cancer Lett.* **1997**, *114*, 71–74.
(32) Romani, S.; Pinnavaia, G. G.; Dalla Rosa, M. Influence of roasting levels on ochratoxin A content in coffee. *J. Agric. Food Chem.* **2003**, *51*, 5168–5171.
(33) Blanc, M.; Pittet, A.; Munoz-Box, R.; Viani, R. Behavior of ochratoxin A during green coffee roasting and soluble coffee manufacture. *J. Agric. Food Chem.* **1998**, *46*, 673–675.

Received for review July 1, 2007. Revised manuscript received September 24, 2007. Accepted September 24, 2007.

JF071959+