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Ochratoxin A on Green Coffee: Influence of Harvest and Drying Processing Procedures

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Ochratoxin A is a metabolite produced by *Aspergillus* and *Penicillium* species that is nephrotoxic and possibly carcinogenic to humans. The aim of this study was to evaluate ochratoxin A contamination in green coffee obtained by different harvesting and drying operations and from fruits of different ripening stages in order to identify hazards. The research was directed to coffees from the highland area of Rio de Janeiro state (Brazil), which is traded in the domestic market. Twenty-two out of 54 samples contained ochratoxin A at levels ranging from 0.3 to 160 μg/kg. Ochatoxin A contamination levels between different ripe stage fruits were not significant ($P > 0.05$). "Varrição" coffee, consisting of fruits that fell from the tree spontaneously and stayed longer on the ground before being harvested, was the most contaminated. Eleven out of 14 samples of varrição coffee were contaminated. Three out of 10 samples from the northwestern region of the state were positive for ochratoxin at levels ranging from 10.1 to 592 μg/kg. The contaminated samples had in common the fact that they were harvested directly from the soil.

KEYWORDS: Ochratoxin A; critical control points; green coffee processing

INTRODUCTION

Ochratoxin A is a mycotoxin produced by three main species of fungi, *Aspergillus ochraceus*, *Aspergillus carbonarius*, and *Penicillium verrucosum*, with a minor contribution by *Aspergillus niger* (*1*). Coffee is a tropical product, and the most likely fungi involved are yellow-spored *Aspergillus* species typified by *A. ochraceus* and the black *A. niger* and *A. carbonarius* (*2*). It has been observed (*3*, *4*) that in Brazilian coffee from the state of São Paulo, *A. niger* was found most commonly but with a low rate of toxigenic strains while *A. ochraceus* was also common with the highest percentage of toxigenic strains. Ochratoxin A is a potent nephrotoxic agent (*5*, *6*) and has been classified by the International Agency for Research on Cancer (IARC) into the group 2B as possibly carcinogenic to humans (*7*). This mycotoxin is also suspect of being the cause of testicular cancer (*8*). Ochratoxin A has been found in green coffee as well as in roasted coffee (*9*−*12*), although some degradation occurs during the roasting process (*13*) attaining 69% reduction (*14*). The adoption of a preventive quality control system based on identifying and controlling the critical points along the processing chain has been suggested as the way to ensure first the healthiness and second the sensory quality of the coffee (*15*).

Coffee processing to the green bean stage has as its primary objective the dehydration of the bean to a point where biological activity is minimized. Two methods are employed in green coffee processing, the dry and the wet method, but both have peculiarities dependent on the regions, since the procedures are not optimized even in the same country. In the first method, coffee fruit is dried in its whole (cherry) state, with pulp and mucilage, and is more frequently employed in Brazil. The wet method is more sophisticated, as the coffee fruit is dried after removal of the pulp and the mucilage (parchment state).

The coffee obtained by the wet method has been associated with a better quality and is employed when an Arabica smooth coffee is sought (*16*). The main reason for this improved quality is the fact that fermentation occurs in the water, allowing lower temperatures during this step. This results in a low level of undesirable flavor compounds such as the ones resulting from butyric fermentation. Another reason for the improved quality with this method might be because the fruits are separated according to their maturity stage, promoting a more homogeneous drying.

The main aim of this study was to evaluate ochratoxin A contamination in green coffee obtained by two processing procedures, wet and dry. In addition, the influence of the harvest procedure and the degree of fruit ripening was also evaluated in order to identify procedures increasing or decreasing the risk of contamination.

MATERIAL AND METHODS

Experimental Sampling. Samples of green coffee (*Coffea arabica*) were collected over a 6 month period (May−November 1999) from the Bom Jardim area in the highlands of Rio de Janeiro state, Brazil,

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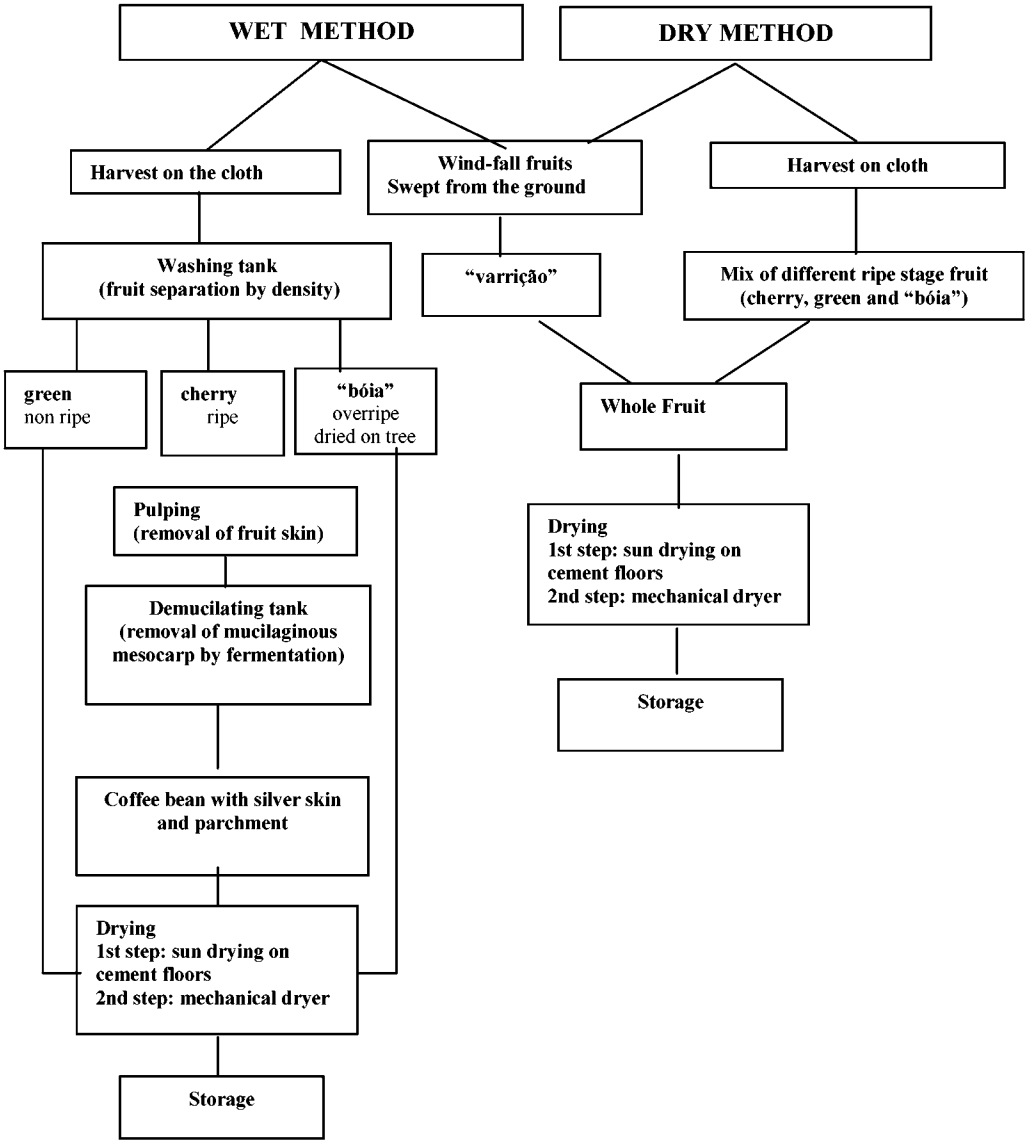

Figure 1. Wet and dry method green coffee processing used in farms A–C from the Highland Region.

which is traded in the domestic market. All samples were collected at the end of the drying process when the moisture was between 11 and 13%. Samples of dried coffee obtained from different ripe stage fruits belonged to the same processed lot. In addition, coffees from Varre Sai area, northwestern region of Rio de Janeiro state, were also sampled and analyzed for the presence of ochratoxin A.

Processing into Green Coffee Employed at the Farms of the Highland Area of Rio de Janeiro State. *Wet Method.* The flowchart of green wet method processing is shown in **Figure 1**. The coffee was harvested on cloth and primarily separated from leaves, wood sticks, and turf soil in a sieve. Afterward, the fruits were washed in tanks where different ripening stages fruits were separated by density. As a result, two fractions of fruits were obtained, the heavier (green and cherry fruits) and the lighter ("bóia"). Cherry ripening stages were defined as green (nonripe), cherry (ripe), and bóia (overripe; dried fruits that remained attached to the tree). The cherry fruits were pulped and transferred to a concrete demucilating tank where they were covered by water, initiating the fermentation of the mucilage. The coffee without mucilage but with the parchment was sun-dried laid on cement floors. The coffee layer was kept at a maximum 5 cm high. The different ripe stage fruits (cherry, green, bóia) and "varrição", which are fruits that fall spontaneously from the tree and stay longer in the soil before being swept up, were dried separately, as shown in **Figure 1**. Varrição coffee was dried as a whole fruit by the dry method. This first step of drying resulted in a coffee grain with a humidity content of 25% and took around 5 days. Mechanical drying (max 55 °C) was used in a second step to bring down the moisture to 11% in around 2−3 days.

Dry Method. Coffee fruit was dried whole, with pulp and mucilage, using solar heat to ca. 44% humidity and then further reduced to 11% in mechanical dryers (**Figure 1**). Coffee obtained this way is called "coco coffee". In dry processing, fruits of different ripening stages are dried together except the varrição type.

Green Coffee Samples from Farms of the Highland Area of Rio de Janeiro State. *Wet Method Processed Coffee.* Twenty-one samples of coffee, obtained from processing different ripening stages fruits (cherry, green, bóia) were obtained from a farm that was called A. Seven of each type including varrição, which is always dry-processed, were collected for comparison, totaling 28 samples.

Dry Method Processed Coffee (Coco). Fourteen samples of coffee were obtained from a farm that was called B. Two type of samples were collected, the coffee obtained from a mix of cherry, green, and bóia harvest on cloth and varrição.

Farms A and B were situated 10 km from each other, and samples were collected always on the same day during the harvesting period. In addition, the procedures for sun drying such as thickness of the layers and frequency of turning the layers were similar. Drying machines also operated at the same temperature in both farms.

Dry Method Processed Organic Coffee (Coco Organic). Six samples of each of the two types of coffee, a mix of cherry, green, and bóia harvest on cloth and varrição, were collected from a farm called C.

Green Coffee Samples from Farms of the Northwestern Area of Rio de Janeiro State. Each of the 10 samples of coco coffee (dry method) was collected from a different small farm. The procedures for obtaining coco coffee were varied. The harvest was done on the cloth and on the ground; drying was done in only one step (in the sun) or in two steps (sun and drying machine); the type of floor where the coffee was laid out to dry in the sun was concrete or ground (compacted soil).

Sample Preparation. The samples were collected in 2 kg paper bags and transported to the laboratory. Samples dried as whole fruit were difficult to grind and were primarily processed using a peeler machine (Pinhalense, Brasil) to remove the husk resulting from drying the fruit with pulp and mucilage. In a second step, samples were ground using a mill (Brabander-CW) equipped with a sieve to select 20 mesh particles. Following grinding, the samples were divided into about 100 g portions, which were kept in the freezer until the time of analysis.

Analysis of Ochratoxin A. Ten grams was analyzed according to the German Official Method, §35 LBMG, 15-00-01 (*17*) with modifications. Thirty milliliters of 2 M HCl in 25 mL of 0.4 M magnesium chloride solution was added to 10 g of ground sample. After the sample was homogenized, 125 mL of toluene was added and the mixture was shaken vigorously for 60 min. The suspension was separated by centrifugation (4000 rpm), and 50 mL of the toluene supernatant was passed through a Sep Pak silica gel column preconditioned with 5 mL of toluene. The column was washed with two 20 mL aliquots of hexane, 20 mL of diethyl ether, and 5 mL of toluene. Ochratoxin A was eluted with 30 mL of toluene/acetic acid (9:1) and was evaporated to dryness at 40 °C. The residue was resuspended into 1 mL of the high-performance liquid chromatography (HPLC) mobile phase and filtered. Detection and quantification were performed by HPLC using a model LC-10 Shimadzu chromatograph equipped with a RF-10AXL fluorescent detector, a 250 mm × 4.6 mm and 5 μm particle size C18 column, and a 1 cm precolumn with the same packing. The isocratic chromatographic conditions were as follows: mobile phase composed of acetonitrile/water/acetic acid (5:4:8:2) pumped at a flow rate of 1 mL/min. The fluorimetric excitation and emission wavelengths were set at 336 and 468 nm, respectively. Ochratoxin A was quantified by comparison with external standards. Ochratoxin A standard was purchased from Sigma (St. Louis, MO). Samples were analyzed in duplicate and, when showing ochratoxin A concentrations higher than 1 μg/kg, were derivatized with BF_3−methanol for identity confirmation. The ones showing less than 1 μg/kg were coinjected with ochratoxin A standards. The detection limit was 0.3 μg/kg, and the quantification limit was 1 μg/kg. The mean recovery for triplicate green coffee blanks spiked at levels of 0.5, 1.0, 5.0, 10.0, and 25.0 μg/kg ochratoxin A was 86%. On the basis of results for spiked samples, the relative standard deviation for repeatability (RSDr) ranged from 1 to 24%.

Statistical Analysis. The experimental data obtained with different ripening stage fruits and varrição coffee were submitted to analysis of variance using a randomized model of factorial experiments (*18*). The comparison of the mean values was done by Tukey test.

RESULTS AND DISCUSSION

Coffee Samples Processed by the Wet Method and Varrição Type from Farm A. As can be seen in **Figure 1**, the coffee fruits are segregated into different ripening stages and dried separately only when processed by the wet method. When the results of coffees obtained with fruits of different ripening stages were compared, no significant differences ($P > 0.01$) were detected, except for the one that was swept from the ground (varrição) (**Table 1**). With the exception of one bóia coffee, ochratoxin A was not detected at levels above 1.0 μg/kg (**Figure 2**). The mean value of ochratoxin A found in bóia coffee was 1.3 μg/kg (range n.d., 8.8 μg/kg), whereas it was 21.5 μg/kg (range n.d., 73.7 μg/kg) in varrição coffee.

The great advantage of the wet method as compared to the dry method is that the fruits at different ripening stages are dried separately, which allows a better control of this step. The cherries are the only coffee fruits that have the mucilage removed by fermentation under water before being dried. Coffee obtained from cherry fruits processed in this way is considered the best in flavor. However, under the conditions of our experiment, the presence of the mucilage during drying did not influence the contamination with ocratoxin A, as the levels of contamination of green and bóia fruits, which are dried as a whole fruit, were not significantly different ($P > 0.05$) from cherry coffee (**Table 1**).

Table 1. Ochratoxin A Contamination of Coffees Obtained from Different Ripe Stage Fruits (Green, Cherry, and Boia) Harvested on Cloth and Varrição (Picked from the Soil)

ripe stage	range of ochratoxin A (μg/kg)	mean value of ochratoxin A (μg kg^{-1})[a]
varrição	ND–73.7	22.5 a
cherry	ND–0.8	0.3 b
bóia	ND–8.8	1.3 b
green	ND–1.0	0.0 b

[a] Means of seven samples of each type collected monthly from May to November. The means followed by different letters differ at a level of significance of 5%; ND, not detected (detection limit of 0.3 μg/kg).

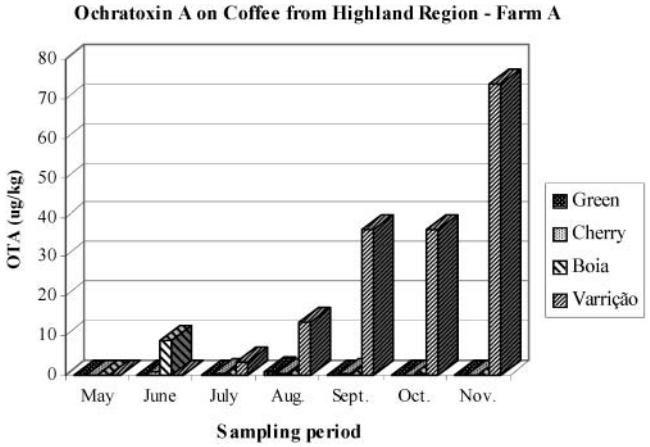

Figure 2. Ochratoxin A contamination of wet processed green coffee obtained from different ripe stages fruits harvest on cloth as compared to coffee swept from the ground (varrição).

In contrast, the varrição coffee, which is swept from the ground and dry-processed, was highly contaminated. From the safety point of view, the varrição presents a serious risk of ochratoxin A contamination. The higher levels of ochratoxin A may be due to a prolonged contact of the fruits with the soil, favoring the colonization by fungi and production of toxin at levels dependent on the weather conditions and the length time that the fruits remain in contact with soil before being harvested and dried. It was observed that the varrição samples from the end of harvest season were more contaminated (**Figure 2**, **Table 2**). This was related to the longer period of time that the fruits were left on the ground before being harvested since, by this time, a large number of the temporary workers were dismissed. According to Krug (*19*), the longer the time the fruits remained on the ground, the greater the incidence of the fungi was and the worse the organoleptical quality of the product was.

Coffee Samples Processed by the Dry Method (Coco Coffee). Fourteen samples from farm B were analyzed for ochratoxin A. Among 13 samples harvested on cloth, only one sample was contaminated with ochratoxin A at a level of 1.0 μg/kg (**Table 2**). Unfortunately, varrição coffee was not available at farm B from monthly collections during the harvest season, but the single sample obtained was contaminated with ochratoxin A at levels as high as 160 μg/kg. As observed on

Table 2. Levels of Ochratoxin A Found in Dry Processed Coffee from Highland Region

sampling period	no. of samples	harvest	ochratoxin A (μg/kg) and SD
		farm B	
May	1	cloth	ND[a]
	2	cloth	ND[a]
June	3	cloth	1.0 ± 0.12
	4	cloth	ND[a]
July	5	cloth	ND[a]
	6	cloth	ND[a]
August	7	cloth	ND[a]
	8	cloth	ND[a]
September	9	cloth	ND[a]
	10	varrição	160 ± 0.02
October	11	cloth	ND[a]
	12	cloth	ND[a]
November	13	cloth	ND[a]
	14	cloth	ND[a]
		farm C—organic coffee	
June	1	cloth	0,4
	2	varrição	46.9 ± 0.29
July	3	cloth	0.3
	4	varrição	3.6 ± 0.12
August	5	cloth	0.3
	6	varrição	13.7 ± 0.32
September	7	cloth	ND[a]
	8	varrição	34.1 ± 0.66
October	9	cloth	ND[a]
	10	varrição	28.4 ± 0.65
November	11	cloth	ND[a]
	12	varrição	71.6 ± 0.37

[a] ND, not detected (detection limit of 0.3 μg/kg).

Table 3. Levels of Ochratoxin A Found in Dry Processed Coffee from Northwestern Region, Harvested and Dried in Different Ways at Different Small Farms

farm/ sample	harvest	drying	ochratoxin A (μg/kg) and SD
D	ground	mechanical drier	15.4 ± 0.08
E	cloth	cement floor	ND[a]
F	cloth	earth floor/mechanical drier	ND[a]
G	cloth	earth floor	ND[a]
H	cloth	earth floor	ND[a]
I	cloth	mechanical drier	ND[a]
J	ground	earth floor	563.3 ± 0.95
K	ground	cement floor	10.1 ± 0.14
L	cloth	cement floor	ND[a]
M	cloth	cement floor	ND[a]

[a] ND, not detected (detection limit of 0.3 μg/kg).

farm A, the harvest on cloth was the most important factor to prevent contamination with ochratoxin A.

The contamination of dry-processed organically cultivated coffee supplied by farm C is shown in **Table 2**. Among 12 samples of organic coffee, the varrição type was once again the most contamined. Varrição ochratoxin A levels ranged from 3.6 to 71.6 μg/kg, with a mean value of 33.0 μg/kg.

Coffee processed by the dry method from the northwestern region was also evaluated for the presence of ochratoxin A as shown in **Table 3**. Each sample came from a different small farm that used only the dry method. The agricultural practices on the farms from these regions were poor, and the process of harvest and drying directly on the ground is still common. The contaminated samples had in common the fact that they were harvested directly from the soil. One of the samples had more than 100 times the acceptable limit value (563.3 μg/kg). This sample was not only harvested directly from the ground but was also dried directly on compacted soil floors. Lower levels were found with the other two samples that despite being harvested from the ground, were dried on cement floors (10.1 μg/kg) or by mechanical drying (15.4 μg/kg). This suggests that the type of floor is important in preventing ochratoxin A contamination, especially when the drying is done in only one step.

Bucheli et al. (*20*) observed that sun drying of coffee cherries in Thailand consistently let to ochratoxin A formation independently of whether cherries were placed on concrete, on bamboo tables, or on the ground. In the present research, it was found that the choice of drying yard might be one important factor in preventing contamination with ochratoxin A, but without doubt, the most critical operation is the harvest procedure. The soil under the coffee trees was found to be heavily contamined with *A. ochraceus* (*21*). Varrição coffee was dried over concrete floors and in a mechanical drier and was consistently contaminated with ochratoxin, because it was already inoculated with the fungi from the soil when harvested.

There are at least four critical points where contamination with ochratoxigenic fungi and ochratoxin A production might occur as follows: (i) before harvesting, which is related to a decline in plant defenses due to a stress condition; (ii) during the harvest, when the harvest is directly from the ground, because of the high contamination of ochratoxigenic fungi in the soil or the fruits fall spontaneously from the tree and stay in contact longer with the soil, allowing not only inoculation but also fungal growth; (iii) from the drying area, due to an unsuitable drying process, which allows the ochratoxigenic fungi to proliferate, or if realized directly on the ground through further inoculation of fungi; and (iv) in the storage due to a remoistening.

Bucheli et al. (*22*) reported that the existing tropical storage conditions of Thailand did not significantly affect green coffee quality, as they found no indications for the presence and growth of ochratoxin A producing fungi or the production of ochratoxin upon storage. Taniwaki et al. (*3*), studying coffees from the state of São Paulo, Brazil, concluded that the infection and toxin formation before harvest are not significant. Average infection rates for cherries taken from trees were very low but were higher in fruit taken from the ground, from the drying yard, and from storage, indicating that infection mostly occurred after harvest, and the fungal sources were likely to be soil, equipment, and drying yard surfaces.

All samples were collected at the end of drying process, when the moisture was between 11 and 13%, just before going to storage in the barns. With the exception of one bóia coffee, the samples contamined with ochratoxin A levels higher than 4 μg/kg had in common the fact that they were harvested from the soil. Therefore, even considering that ochratoxin A can be produced on fruits from the tree, it is evident that the contact with the soil is the main critical point.

It was concluded that varrição, as well as coffee harvesting and drying directly on the ground, should not be used. Contact of the coffee with the soil provided inoculation with ochratoxin producing mold. In the case of varrição, it should be necessary, at least, to shorten the time that the fruits remain on the soil before they are harvested. Varrição coffee represents an important source of income and is usually not rejected but mixed with other coffee defects, including husks, in good quality coffee. However, the results of this work were conclusive upon the importance of harvesting on cloth to ensure the healthiness of the product. In addition, harvesting on cloth will save labor in sieving foreign matter from coffee.

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