

# Behavior of Ochratoxin A during Green Coffee Roasting and Soluble Coffee Manufacture

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As considerable inconsistencies are found in the literature regarding the influence of roasting and subsequent operations on the ochratoxin A (OTA) content of green coffee, experiments were undertaken to assess the evolution of OTA along an industrial soluble coffee manufacturing line. Both the variability and the amount of OTA naturally present in a lot of Thai Robusta green coffee were drastically reduced during soluble coffee manufacture. A small proportion of OTA was eliminated during green coffee cleaning, but the most significant reduction took place during roasting. The roast and ground coffee contained only 16% of the OTA originally present in the green coffee. Two phenomena are responsible for the elimination of OTA during roasting: a thermal degradation and a removal with chaff. Thermal degradation is the most important route of elimination, with <20% accounted for by the chaff. A further 20% reduction was observed during soluble coffee manufacture, so that the powder contained only 13% of the OTA initially present in the green beans.

**Keywords:** *Ochratoxin A; green coffee; roasted coffee; soluble coffee; degradation*

## INTRODUCTION

Ochratoxin A (OTA) is a nephrotoxic and nephrocarcinogenic mycotoxin produced by several fungal species from the *Aspergillus* genus and by *Penicillium verrucosum*. It is most commonly found in cereals and cereal products, although a wide range of commodities have been reported as containing the toxin (Pohland et al., 1992; European Commission, 1997).

The natural occurrence of OTA in green coffee beans has been reported by several authors in concentrations ranging between 0.2 and 360  $\mu\text{g}/\text{kg}$  (Levi et al., 1974; Levi, 1980; Norton et al., 1982; Cantafora et al., 1983; Tsubouchi et al., 1984; Micco et al., 1989; Studer-Rohr et al., 1994).

However, considerable inconsistencies are found in the literature regarding the influence of roasting and subsequent operations on the OTA content of coffee (Viani, 1996). Levi et al. (1974) have shown that experimental roasting under conditions simulating those of the typical roasting operation (20 min at  $200 \pm 5^\circ\text{C}$ ) destroyed 77–87% of the OTA added to green coffee. Gallaz and Stalder (1976) also examined the effect of experimental roasting and found that normal roasting destroyed 80–90% of the toxin. Cantafora et al. (1983) could not detect any OTA after roasting two green coffee samples naturally contaminated with 3.8 and 23.0 ppb of OTA, while Micco et al. (1989) reported a 90–100% reduction of the OTA level following the roasting of two green coffee samples naturally contaminated with 4.0 and 8.6  $\mu\text{g}/\text{kg}$ .

On the other hand, Tsubouchi et al. (1987) reported that OTA in artificially contaminated green coffee beans was only slightly reduced (0–12%) by heat treatment

at  $200^\circ\text{C}$  for 10–20 min and that almost all of the toxin was infused into the coffee decoction when the roasted samples were ground and extracted with boiling water. This is broadly in agreement with results published by Studer-Rohr et al. (1994) but in contradiction with those of Micco et al. (1989), who examined the OTA content in beverages prepared from artificially contaminated coffee and found no residues of OTA.

It has been suggested that the discrepancy between these findings may be due to differences in the method of introducing the toxin into the ground beans (Tsubouchi et al., 1987) or the fact that analytical methods employed until recently would not have been sufficiently sensitive and selective to detect low levels of OTA in roasted coffee beans and coffee decoctions (Pittet et al., 1996).

The objective of the present work was to assess the evolution of OTA along an industrial soluble coffee manufacturing line, taking advantage of a sensitive immunoaffinity column/HPLC procedure that has been developed in our laboratory (Pittet et al., 1996).

## EXPERIMENTAL PROCEDURES

**Raw Material.** Experiments were conducted with a lot of Thai Robusta green coffee, which consisted of 280 bags of 60 kg each. This lot had been previously identified as containing a relatively high amount of OTA. To determine the mean contamination level of this lot and study the distribution of OTA, a total of 50 bags were randomly selected. From each bag, a composite sample of  $\sim 300$  g of green coffee beans was taken for OTA analysis. Half of the beans were withdrawn from the center of the bag, while the rest was made up of four equal portions taken, respectively, from the top, bottom, right side, and left side of the bag.

**Green Coffee Cleaning.** This operation consisted of the elimination of foreign matter (such as stones and plant fragments) as well as part of the silverskin by density segregation and air suction, respectively.

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**Table 1. Distribution of Ochratoxin A in a Lot of Naturally Contaminated Thai Robusta Green Coffee**

individual results ( $n = 50$ )	6.0	5.9	7.8	10.0	5.0
	7.3	6.2	5.8	6.2	4.6
	8.4	6.1	4.7	6.1	7.9
	6.0	8.8	11.6	4.7	7.4
	7.3	7.3	9.9	4.5	6.8
	6.7	5.4	7.9	4.2	7.4
	6.7	7.8	8.5	6.0	5.4
	22.1	5.4	4.0	9.1	6.3
	9.6	11.4	5.4	6.7	8.7
	11.0	5.3	6.5	5.5	8.0
range of contamination ( $\mu\text{g}/\text{kg}$ )	4.0–22.1				
mean ( $\mu\text{g}/\text{kg}$ )	7.3				
median ( $\mu\text{g}/\text{kg}$ )	6.7				
SD ( $\mu\text{g}/\text{kg}$ )	2.8				
RSD (%)	39				

**Roasting.** A Probat roaster RN 1000 was used. The moisture level of green coffee was 10.0%, and the amount per charge was 120 kg with a roasting cycle of 14 min. The air temperature was selected for a medium roasting level. The final temperature was 223 °C with total losses of 16.4%. Samples of chaff eliminated during roasting were collected. The roasted coffee had a residual moisture of 1.4%.

**Grinding.** The roasted coffee was ground using a Matsubo grinder Crak-U-Lator model GRN 4531.

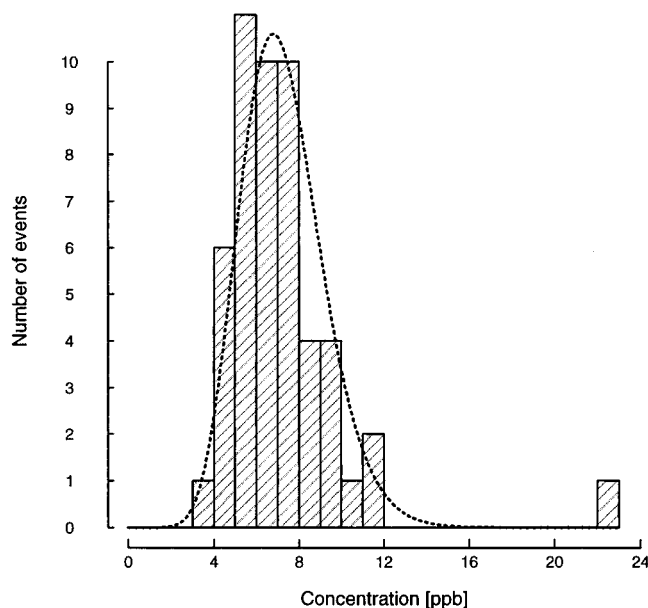
**Extraction.** Typical average conditions such as described in the technical literature (Sivetz and Foote, 1963; Rothfos, 1986; Viani, 1986) were applied for extraction. Each pressure vessel used contained 60 kg of roast and ground coffee. The inlet temperature of hot water was 180 °C. The extraction stream was operated countercurrently. The cycle time and the flow rate through the extractors were such that the extraction yield was 45%, calculated on the basis of roast and ground coffee. The extract was then concentrated and spray-dried.

**Sampling Scheme.** The following samples were collected during the successive operations: green coffee before cleaning (50 samples), green coffee after cleaning (6), cleaning residue (1 representative sample, duplicate analysis), roasted coffee (6), removed chaff (1 representative sample, duplicate analysis), roast and ground coffee (8), spent grounds (3), and soluble coffee (3). It should be mentioned that, for technical reasons, the cleaning residue and removed chaff could not be recovered quantitatively; hence, the total amount of material could only be estimated.

**OTA Analyses.** Each sample was analyzed for OTA according to the high-performance liquid chromatography (HPLC) method of Pittet et al. (1996), which may be summarized as follows. Finely ground coffee beans (25 g) or soluble coffee (5 g) was blended with, respectively, 500 or 100 mL of methanol/3% aqueous sodium hydrogen carbonate (50:50) and filtered. An aliquot of 4 mL of filtrate was diluted to 100 mL with phosphate-buffered saline (PBS), and the whole diluted extract was applied to a Vicam OchraTest immunoaffinity column, at a slow steady flow rate of 2–3 mL/min. After the column was washed with 10 mL of distilled water, OTA was eluted with 4 mL of methanol. The eluate was then evaporated to dryness under a stream of nitrogen at 40 °C, and the residue was redissolved in 150  $\mu\text{L}$  of 45% acetonitrile–55% 4 mM sodium acetate/acetic acid (19:1) prior to separation on a reversed-phase HPLC system equipped with a fluorescence detector operated at an excitation wavelength of 330 nm and an emission wavelength of 470 nm.

## RESULTS AND DISCUSSION

**Distribution of OTA in Naturally Contaminated Green Coffee.** The concentrations of OTA found in each individual bag of uncleaned green coffee are shown in Table 1. The range of contamination was 4.0–22.1  $\mu\text{g}/\text{kg}$ , with an average concentration of 7.3  $\mu\text{g}/\text{kg}$  and a median of 6.7  $\mu\text{g}/\text{kg}$ . The variance is 8.0, which corre-

**Figure 1.** Histogram of OTA data obtained on a lot of naturally contaminated Thai Robusta green coffee (50 samples). The dotted line represents the negative binomial distribution.**Table 2. Fate of Ochratoxin A along a Soluble Coffee Manufacturing Line**

operation	material	ochratoxin A content ( $\mu\text{g}/\text{kg}$ )			
		individual results		mean	
reception	uncleaned green coffee	see Table 1			
cleaning	cleaned green coffee	4.3	6.9	9.0	6.8
	cleaning residue	68.2	66.7	4.2	67.5
roasting	roasted coffee	1.3	1.0	1.2	1.4
	removed chaff	1.5	1.6	1.9	
grinding	roast and ground coffee	153.5	156.3		154.9
		2.1	1.2	1.2	1.4
		1.2	1.3	1.1	
		1.6	1.1		
extraction	spent grounds	<0.2 <sup>a</sup>	<0.2	<0.2	<0.2
spray-drying	soluble coffee <sup>b</sup>	2.4	2.7	2.5	2.5

<sup>a</sup> Corresponds to the limit of detection of the analytical method used. <sup>b</sup> Extraction yield: on average 1 kg of roast and ground coffee gave 450 g of soluble coffee.

sponds to a standard deviation (SD) of roughly 2.8  $\mu\text{g}/\text{kg}$ . This clearly gives an asymmetric distribution, as illustrated in Figure 1.

To carefully assess the evolution of OTA during processing, it is important to first study the distribution of the toxin in green beans, even when relatively small sample sizes are used as in the present work. For this purpose, an OTA contamination at bean level similar to that of aflatoxins in peanuts was considered as a working hypothesis, and the results presented in Table 1 were used to test whether a negative binomial function might be appropriate to characterize the distribution of OTA. This was done by applying the model of Whitaker et al. (1994), which had been developed by the Food and Agriculture Organization (FAO) to evaluate the distribution of aflatoxins in peanuts (FAO, 1993). A test of goodness of fit ( $\chi^2$  test,  $p < 0.52$ ) turned out in favor of the negative binomial model, which is illustrated by the dotted line superimposed on our experimental data in Figure 1.

**Fate of OTA during Soluble Coffee Manufacture.** The results are presented in Tables 2 and 3. Although a small proportion of OTA was eliminated during the cleaning of green coffee, the most significant reduction

**Table 3. Overall Ochratoxin A Reduction during Soluble Coffee Manufacture**

material	quantity (kg)	mean OTA concn ( $\mu\text{g}/\text{kg}$ )	total amt of OTA ( $\mu\text{g}$ )	residual OTA (%)
uncleaned green coffee	3000	7.3	21900	100
cleaned green coffee		6.8		
roasted coffee	2490	1.4	3486	16
soluble coffee	1120	2.5	2800	13
chaff at 0.3%	7.5	154.9	1157	5
chaff at 0.5%	12.5	154.9	1929	9
chaff at 1.0%	24.9	154.9	3857	18

took place during roasting, when OTA was either eliminated with chaff or destroyed. As it was not possible to accurately determine the proportion of chaff removed during roasting, the following hypotheses were made to estimate the extents of OTA elimination by this route (see Table 3): low (0.3% chaff), intermediate (0.5% chaff), and high (1.0% chaff). Even in the case of 1.0% chaff, the total amount of OTA that would have been removed with chaff remains small (<20%) in comparison with the overall reduction in OTA content observed during the roasting process. Therefore, the OTA degradation during roasting can be attributed to both thermal destruction and chaff removal. A further 20% reduction was observed during soluble coffee manufacture, while only traces of toxin (<0.2  $\mu\text{g}/\text{kg}$ ) were detected in the spent grounds. This additional reduction might be explained partly by the residual content of OTA in spent grounds, partly by the analytical variability of the method used, and possibly also by a limited hydrolysis of OTA during the high temperature step of coffee solubilization. These results are consistent with brewing experiments previously undertaken in our laboratory, where ~80% of OTA initially present in roast and ground coffee was found to be transferred into the cup (A. Pittet, unpublished results), and confirm that OTA is easily solubilized by hot water.

In conclusion, both the variability and the amount of OTA present in green coffee are drastically reduced during soluble coffee manufacture. A small proportion of OTA is eliminated during green coffee cleaning, but the most significant reduction takes place during roasting. With the particular green coffee used in these trials, the roast and ground coffee contained only a residual 16% of the OTA originally present in the green beans. Taking into account an extraction yield of ~80% during brewing, a 150 mL cup of coffee prepared from this roast and ground coffee at a strength of 50 g/L would then contribute an OTA intake of ~8 ng. The residual 13% OTA present in the final powder indicates a further reduction during soluble coffee manufacture, and a cup of coffee prepared from 2 g of powder would contain ~5 ng of OTA. The results of this study therefore indicate that both roast and ground coffee and soluble coffee are only minor sources of OTA in the diet, even when prepared from relatively highly contaminated green beans.

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Received for review September 8, 1997. Revised manuscript received November 24, 1997. Accepted November 25, 1997.

JF9707703