Effect of Roasting Conditions on Reduction of Ochratoxin A in Coffee

Gerrit H. D. van der Stegen,* Paulus J. M. Essens, and Joost van der Lijn

Sara Lee Douwe Egberts, Vleutensevaart 35, NL-3532 AD Utrecht, The Netherlands

A commercial lot of green coffee, naturally contaminated with ochratoxin A (OTA), was roasted under various conditions, and the effects on its final OTA content were determined. Precautions were taken in sampling the coffee to cope with OTA inhomogeneity. The roasting conditions were kept within the range of commercial practice. Roasting time was varied from 2.5 to 10 min, and the roast color varied from light medium to dark. The differences in OTA reduction between the different levels of roasting times and colors did not reach statistical significance. However, for all roasting conditions, the reduction was highly significant, 69% reduction over the combined results. In total, nine studies by various authors about OTA reduction during coffee roasting are now available. Seven out of these nine reported that the relevant range of OTA reductions was between 69 and 96%. Among these seven,are all four studies that reported using naturally contaminated beans, a sampling procedure adapted to mycotoxin inhomogeneity, and roasting conditions within the range of actual practice. Three different explanations are available for this reduction: physical removal of OTA with chaff, isomerization at the C-3 position into another diastereomer, and thermal degradation with possible involvement of moisture. All three explanations may play a partial role in the OTA reduction during coffee roasting.

Keywords: *Ochratoxin A; mycotoxin; roasting; coffee; reduction*

INTRODUCTION

Occurrence of ochratoxin A (OTA) in coffee brews was first reported in 1988 (*1*). Before that time, it was generally thought that OTA decomposed during roasting (*2*−*4*). Since then, more experiments about the fate of OTA during the roasting of coffee were published (*5*−*9*). The way of contamination of the green coffee in the eight studies published so far (*2*−*9*) varied over “naturally contaminated”, “artificially inoculated”, and “sprayed with OTA”. Roasting conditions, if specified, varied from conditions clearly outside actual coffee roasting practice to roasting experiments on a commercial scale. From those using naturally contaminated green coffee, only three (refs *6*, *8*, and *9*) reported to have sampled their green coffee taking into account the inhomogeneity of mycotoxin contamination. Over these published studies, the results varied from almost none to almost complete reduction of OTA during roasting. Our study aimed to give further insight into the effect of roasting conditions on the reduction of OTA during roasting.

MATERIALS AND METHODS

Green Coffee. Experiments in this study were carried out using one lot of 600 kg of OTA-containing Robusta coffee (*Coffea canephora*) from Cote d'Ivoire.

Homogenization. To cope with OTA inhomogeneity in green coffee, the lot was well mixed in a Nauta mixer in order to make equal batches out of it as much as possible. The homogenized lot was divided into batches, 5−15 kg in size, for roasting and OTA determination in the green coffee.

* Corresponding author e-mail: gvanderstegen@saralee-de.com. Telephone: +31 30 297 2836. Fax: +31 30 297 2745.

Table 1. Roasting Conditions

batch size (kg)	roasting air temp (°C)	roasting time (min)	temp in coffee bed before cooling (°C)	moisture content (%)	color value ground coffee
5	470	2.5	217	2.3	60 (light medium)
5	490	2.5	228	2.0	40 (dark)
10	490	4	228	2.3	50 (dark medium)
15	400	10	224	2.2	60 (light medium)
15	425	10	240	2.3	40 (dark)

Grinding of Green Coffee for OTA Determination. Ten batches of 6 kg each were ground in a Condux toothed disk mill. The ground coffee of each batch was again well mixed for homogenization. From the homogenized mixtures, 250 g was taken for further analysis.

Roasting Conditions. Roasting was carried out in a Gothot RN 100 pilot size roaster. Roasting times used were as follows: 2.5, 4, and 10 min. Variations in degree of roast were light/medium roast (color value 60), dark/medium roast (color value 50), and dark roast (color value 40) (color values determined using an Agtron E5c color meter after grinding to 0.4 mm average particle size). Conditions needed for the desired color were determined in a few trial runs, and then five or six batches were produced under the same conditions for further evaluation. Roasting conditions are indicated in Table 1. The roasting was stopped in the usual manner by spraying of cooling water, followed by discharging onto a sieve plate and blowing of ambient cooling air. To avoid sequential effects, the order of the experiments has been randomized.

Grinding. The resulting batches of roasted coffee (varying batch size) were ground in a Trabattoni Isidoro disk mill to an average particle size of approximately 0.4 mm. The ground coffee of each batch was well mixed for homogenization. From each homogenized mixture, 250 g was taken for further analysis.

OTA Determination. The method of analysis was similar to the one published by Pittet et al. (*10*). Finely ground coffee (30 g) was extracted with 200 mL of methanol/3% aqueous sodium hydrogen carbonate (50:50) for 30 min in a closed bottle

10.1021/jf0105586 CCC: $20.00 © 2001 American Chemical Society
Published on Web 09/28/2001

Table 2. Average OTA Contents before and after Roasting

roasting time (min)	color	no. of samples	batch size (kg)	av OTA content (ng/g)
	Green Coffee			
		10	6	4.9
	Roasted Coffee			
2.5	60 (light medium)	6	5	1.6
2.5	40 (dark)	6	5	1.8
4	50 (dark medium)	6	10	1.3
10	60 (light medium)	5	15	2.3
10	40 (dark)	5	15	0.8

Table 3. Monovariate Statistics for the OTA Content in Samples from One Lot of OTA-Containing Coffee

green coffee		roasted coffee	
N	10	*N*	28
mean	4.9	mean	1.5
SD	3.2	SD	1.2
skewness	2.7	skewness	0.6

in an ultrasonic bath. The extract was centrifuged at 7500 rpm, and 5 mL of the supernatant was diluted with 80 mL of phosphate-buffered saline (PBS). A total of 10 mL of the PBS diluted extract was passed over an immunoaffinity column at a rate of 1 mL/min. After the column was washed with 15 mL of demineralized water at a rate of 2 mL/min, it was drained till dryness. A total of 4 mL of methanol was used to elute the OTA from the column at a rate of 0.5 mL/min. (First methanol was allowed to soak into the column for 5 min.) The methanol eluate was evaporated to dryness under N_2, the residue was redissolved in 200 μL of a water–acetonitrile–methanol mixture (50:32:18), and 50 μL thereof was injected into the HPLC. Chromatography was done on a reverse-phase column with an aqueous acetate buffer acetonitrile eluent and fluorescence detection with excitation at 330 nm and detection at 460 nm. Spiked with 3.1 ng/g (ppb), the recovery was 84%, and for a sample with 2.4 ppb, the CV was 11%. The detection limit was 0.5 ppb, and for calculations, the results below the detection limit were taken as being 0.25 ppb.

RESULTS

The effect of the roasting on the average OTA content is given in Table 2. The effect of the roasting time and degree of roast (color) on the OTA content was studied by analysis of variance. In view of the nonnormal distribution of the individual results, this analysis was carried out on the ranks of the individual results. No statistically significant differences between the levels of the applied roasting time and color were found.

In view of the absence of major effects between the roasting conditions, the results for all roasted products have been pooled and compared to those of the untreated green coffee. Monovariate statistics of the two categories are given in Table 3. Ranges are given in Figure 1.

The results have been compared using several nonparametric difference tests. All showed a highly significant (Table 4) reduction of OTA during roasting.

DISCUSSION

During the last 25 years, eight previous studies were published about the reduction of OTA during coffee roasting (*2–9*). Ways in which the green coffee got OTA contaminated and in which the green coffee was sampled for OTA analysis varied substantially among these studies. Mycotoxins may be highly nonnormally distributed over lots, as can be seen from Table 3. For this reason, an adequate sampling procedure including the

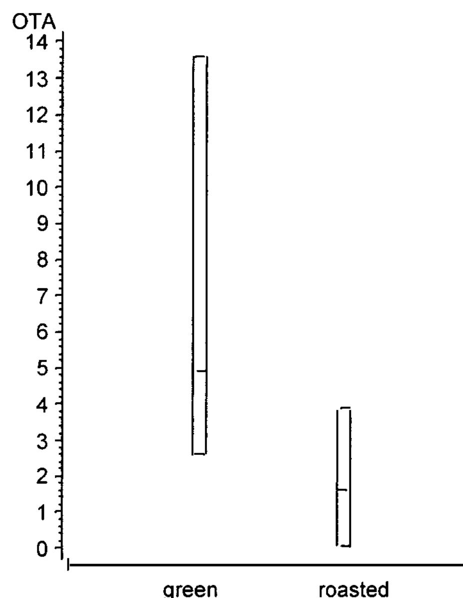

Figure 1. Ranges for OTA contents nonroasted and roasted.

Table 4. Results of the Nonparametric Statistical Tests

```
Wilcoxon 2-Sample Test (Normal Approximation)
                    (with Continuity Correction of .5)
                    S =  316        Z =  4.01        Prob > |Z| = 0.0001
                    T-Test Approx. Significance = 0.0003
Kruskal-Wallis Test (Chi-Square Approximation)
                    CHISQ =  16.1        DF =  1          Prob > CHISQ = 0.0001
Median 2-Sample Test (Normal Approximation)
                    S =  10        Z =  3.64        Prob > |Z| = 0.0003
Median 1-Way Analysis (Chi-Square Approximation)
                    CHISQ =  13.2        DF =  1          Prob > CHISQ = 0.0003
Van der Waerden 2-Sample Test (Normal Approximation)
                    S =  10.1        Z =  4.03        Prob > |Z| = 0.0001
Van der Waerden 1-Way Analysis (Chi-Square Approximation)
                    CHISQ =  16.2        DF =  1          Prob > CHISQ = 0.0001
```

grinding and homogenization of large samples is always required. Roasting varied in these studies from clearly outside the common roasting practice to roasting in commercial scale equipment.

Only four of the now available studies (including this one) used naturally contaminated green coffee *and* reported a sampling procedure in some way adapted to mycotoxin inhomogeneity *and* reported roasting conditions within the range of normal commercial practice. These four studies found consistently high percentages of OTA reduction during roasting (see Table 5).

In the present study, a reduction of the OTA content by 69% was observed. This percentage is slightly lower than what was observed in the other three studies. Such a difference might be due to the choice for mechanical mixing of the green coffee to compensate for OTA inhomogeneity, with the possible consequence of some separation of silverskins (chaff) before roasting.

Our study did not show statistically different effects for levels of roasting times or roasting colors. However, the highest OTA reduction was seen for the combination of long plus dark roasting, which might indicate that statistical significance could be reached in the interaction of longer times and darker colors.

Another observation is that the distribution after roasting tends to be less skewed, apparently the highest contamination is most effected. Hence, in practice there will be variation in the final degree of reduction, not only as a result of statistical fluctuations but also as a

Table 5. OTA Reduction during Coffee Roasting

coffee type	OTA in green coffee (ppb)	OTA in roasted coffee (ppb)	OTA reduction at roasting (%)	reference
Uganda Robusta	0.9	0.63	30	Wilkens et al. (*9*)
Conillon	4	0.3	90	Micco et al. (*6*)
Ivory Coast Robusta	4.9	1.5	69	this study
Thai Robusta	7.3	1.4	84	Blanc et al. (*8*)
Zaire	8.6	0.2	96	Micco et al. (*6*)
Ivory Coast Robusta	9.91	2.11	79	Wilkens et al. (*9*)
Ethiopia Arabica	18.4	1.9	89	Wilkens et al. (*9*)

function of the degree of contamination. This effect was confirmed by the results in Table 5 and more specifically those of Wilkens and Jörissen (*9*).

Of the five studies that did not meet the above criteria, three reported 80% or more reduction (*2*−*4*), and two reported hardly any OTA reduction (refs *5* and *7*) during coffee roasting. Tsubouchi et al. (*5*) contaminated beans by inoculation with *A. ochraceus*. The contaminated beans were kept in an open stainless steel dish, which was heated at 200 °C using an air heater for 10−20 min. These conditions will result in temperatures in the beans substantially below the temperatures reached in actual roasting practice (for comparison, see Table 3). Studer-Rohr et al. (*7*) split naturally contaminated samples, reportedly being spoiled, of up to 1 kg into two parts: one being roasted and the other being directly analyzed. For samples of coffee contaminated by inoculation, they reported pre-drying for 2 h at 80 °C. In view of the very short roasting times (100−190 s) also, it is questionable whether adequate roasting has occurred, even though roasting took place in a commercial small roasting machine. Furthermore, the reported variability for the roasting results contained the repeatability of the analysis and the extraction but not the (often very large) variability of the sampling.

Several of these studies mention physical removal of OTA with the silverskins (chaff) as a possible mechanism for the observed reduction, but their data show that this can only partially be the explanation (*8*). Another possible explanation was given by Studer-Rohr et al. (*7*), who showed partial isomerization of OTA at the C-3 position into a less toxic diastereomer. However, this also seems to be only a partial explanation. A third explanation might come from a study about thermostability of OTA in wheat under two moisture conditions (*11*). Whereas Studer-Rohr et al. (*7*) showed that pure dry OTA is quite thermostable and only isomerizes slowly, Boudra et al. (*11*) showed substantial effects of the presence of moisture on the reduction of OTA when heating contaminated wheat. The role of moisture could also explain the wide variation of OTA reduction at roasting, as observed in the other studies applying quite unusual roasting conditions, such as application of predrying or heating at relatively low temperatures in addition to sampling procedures not adapted to mycotoxin inhomogeneity.

LITERATURE CITED

(1) Tsubouchi, H.; Terada, H.; Yamamoto, K.; Sakabe, Y. Ochratoxin A found in commercial roast coffee. *J. Agric. Food Chem.* **1988**, *36*, 540−542.

(2) Levi, C. P.; Trenk, H. L.; Mohr, H. K. Study of the occurrence of ochratoxin A in green coffee beans. *J. Assoc. Off. Anal. Chem.* **1974**, *57*, 866−870.

(3) Gallaz, L.; Stalder, R. Ochratoxin A in Kaffee. *Chem. Mikrobiol. Technol. Lebensm.* **1976**, *4*, 147−149.

(4) Cantafora, A.; Grossi, M.; Miraglia, M.; Benelli, L. Determination of ochratoxin A in coffee beans using reversed-phase high performance liquid chromatography. *Riv. Soc. Ital. Sci. Aliment.* **1983**, *12*, 103−108.

(5) Tsubouchi, H.; Yamamoto, K.; Hisada, K.; Sakabe, Y.; Udagawa, S. Effect of roasting on ochratoxin A level in green coffee beans inoculated with *Aspergillus ochraceus*. *Mycopathologia* **1987**, *97*, 111−115

(6) Micco, C.; Grossi, M.; Miraglia, M.; Brera, C. A study of the contamination by ochratoxin A of green and roasted coffee beans. *Food Addit. Contam.* **1989**, *6*, 333−339.

(7) Studer-Rohr, I.; Dietrich, D. R.; Schlatter, J.; Schlatter, Chr. The Occurrence of Ochratoxin A in Coffee. *Food Chem. Toxicol.* **1995**, *33* (5), 341−355.

(8) 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.

(9) Wilkens, J.; Jörissen, U. Abbau von Ochratoxin A bei der Produktion von Röstkaffee. In *Proceedings 21 Mykotoxin-Workshop Jena, June 7−9, 1999*; Rosner, H., Kielstein, P., Eds.; BGVV: Jena, Germany, 1999; pp 245−251.

(10) Pittet, A.; Tornare, D.; Huggett, A.; Viani, R. Liquid Chromatographic Determination of Ochratoxin A in pure and Adulterated Soluble Coffee Using an Immunoaffinity Column Cleanup Procedure. *J. Agric. Food Chem.* **1996**, *44*, 3564−3569.

(11) Boudra, H.; Le Bars, P.; Le Bars, J. Thermostability of Ochratoxin A in Wheat under Two Moisture Conditions. *Appl. Environ. Microbiol.* **1995**, *March*, 1156−58.

Received for review April 30, 2001. Revised manuscript received July 31, 2001. Accepted August 1, 2001.

JF0105586