Screening on the Occurrence of Ochratoxin A in Green Coffee Beans of Different Origins and Types

Santina Romani*,† Giampiero Sacchetti,† Clemencia Chaves López,† Gian Gaetano Pinnavaia,† and Marco Dalla Rosa‡

Dipartimento di Protezione e Valorizzazione Agro-Alimentare, Sezione di Chimica e Tecnologia degli Alimenti, Università degli Studi di Bologna, Via S. Giacomo 7, 40126 Bologna, Italy, and Dipartimento di Scienze degli Alimenti, Università degli Studi di Udine, Via Marangoni 97, 33100 Udine, Italy

Since to our knowledge no data are available in the literature regarding the influence of green coffee type and origin on ochratoxin A (OTA) content, determinations were carried out in order to assess the level of OTA contamination in green coffee samples of different provenience. A total of 162 samples of green coffee beans from various countries (84 from Africa, 60 from America, and 18 from Asia) were analyzed for OTA. Both the amount and the variability of OTA levels were tested as a function of green coffee provenience. The results showed that 106 of the overall samples were positive for OTA, with concentration ranging from 0 to 48 μg/kg (ppb). In particular, it was possible to verify that African samples were more contaminated with respect to samples of other origin in terms of frequency and level of OTA; the highest concentrations observed were 18 and 48 μg/kg in two samples from The Congo.

Keywords: *Ochratoxin A; screening; green coffee; countries of origin*

INTRODUCTION

Ochratoxin A (OTA), which is produced by several fungal species, can occur in coffee beans both for environmental conditions (climatic conditions, abnormally long storage, and transportation) and for processing conditions (wet or dry process) (Smith et al., 1994; Illy et al., 1995; Viani, 1996).

In the last few years, importer coffee companies and researchers have taken a greater interest in the mycotoxicological quality of the green coffee to estimate the risk for humans imposed by OTA, but insufficient data are available to set regulatory levels based on accurate scientific risk assessment for human and animal health. The European Commission has not yet fixed maximum levels for OTA in coffee, and only has one recommendation (CE 22/12/1998 No. 26), published in the G.U.C.E. No. L7 of January 13, 1999, in which a reference level of 3 μg/kg was suggested to EC Member States. Actually the only UE Member States that apply statutory legal limits for OTA are Italy (8 ppb for green coffee and 4 ppb for final product), Finland (10 ppb), and Greece (20 ppb).

Italy, as do all importer coffee countries, has a reduced possibility to intervene in the sanitary quality of this commodity; a quality that is more uncertain if we consider that the green coffee producing areas are in emerging Third World countries.

The occurrence of OTA in green coffee has been described in a wide range of literature (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; Studer-Rohr, 1995), but to our knowledge no published data are available in the literature regarding the correlation between the type and/or the origin of green coffee and its eventually content on ochratoxin A.

Mislivec et al. (1983) found that there was a greater internal invasion, after disinfection, of toxigenic and other molds in green coffee beans from Asiatic and African countries than in those from Central and South America. Stack et al. (1983), in a work on different mycotoxins production by isolates of *Aspergillus ochraceus* from green coffee beans, could not find firm correlation between toxin production and country of origin of coffee beans.

For a common approach to the control of OTA hazards in green coffee, a discrimination of this mycotoxin presence in green coffee beans from different countries appears to be one of the most important initial steps. The aim of this work was to discriminate for OTA contamination, in terms of frequency and level, several green coffee samples from different countries of origin.

MATERIALS AND METHODS

Raw Material. In total, 162 green coffee samples (weighing about 2 kg), coming from different lots were provided by a coffee company over 1 year. The sampling was conduced from the provider; several aliquots of each sample was taken at different site in each lot (ISO 4072, 1982). Of all the 162 coffee samples used in this trial, 60 were from various countries of Central and South America, 84 were from various countries of Africa, and 18 were from various countries of Asia.

Grinding. Each sample was finely ground using an Officine Vittoria grinder (Bologna, Italy) with conical cutters. The deriving powder was homogenized by stirring to get rid of any contamination difference.

OTA Analyses. OTA analysis was performed on all samples according to the high-performance liquid chromatography (HPLC) method of Nesheim et al. (1992), which may be summarized as follows. The method consists of first extraction

* Corresponding author e-mail: santina@kaiser.alma.unibo.it. Telephone: 0039 51 2099781. Fax: 0039 51 2099782.
† Università degli Studi di Bologna.
‡ Università degli Studi di Udine.

10.1021/jf990783b CCC: $19.00
Published on Web 07/08/2000

Table 1. Overall Results of OTA Screening

origin and type of coffee	no. of samples	no. of positive samples	ochratoxin A level(μg/kg) positive samples min	max	mean	±SD	all samples mean	±SD	no. of samples with ≥3 μg/kg OTA[a] (on overall contaminated)
				Africa					
Zaire	32	32	0.5	12.5	4.9	3.6	4.9	3.6	20
Burundi	1	0							0
Avorio	31	30	0.5	8.3	3.9	1.9	3.8	2.0	20
Uganda	1	1	0.5	0.5	0.5		0.5		0
Etiopia	2	1	4.8	4.8	4.8		2.4	3.4	1
Ghana	3	2	6	1	3.4	2.5	3.4	2.5	2
Congo	7	6	48	5.6	16.6	16.0	14.3	15.9	6
Camerun	7	4	2.5	1	1.7	0.7	1.0	1.0	0
total	84	76	0.5	48	5.2	1.4	4.7	5.9	49
				America					
Santos	32	9	7.7	0.5	2.2	3.1	0.6	1.9	2
Cuba	2	0					0	0	0
Honduras	5	3	0.5	0.5	0.5		0.3	0.3	0
Colombia	9	2	0.5	0.5	0.5		0.1	0.2	0
San Salvador	3	1	0.5	0.5	0.5		0.2	0.3	0
Santo Domingo	1	1	0.6	0.6	0.6		0.6		0
Mexico	1	0					0		0
Costarica	1	0					0		0
Nicaragua	1	1	0.5	0.5	0.5		0.5		0
Guatemala	5	2	2.1	0.1	0.9	1.1	0.5	0.9	0
total	60	19	0.1	7.7	1.3	2.2	0.5	1.4	2
				Asia					
Vietnam	15	9	3.5	0.2	1.4	1.1	1.0	1.0	1
Djimmah	2	2	4.9	0.5	2.7	3.1	2.7	3.1	1
India Cherry	1	0					0		0
total	18	11	0.2	4.9	1.6	1.4	1.1	1.4	2

[a] OTA level of 3 μg/kg was taken as reference value according to the European Commission Recommendation CE 22/12/1998 No. 26.

from 50 g of coffee powder with chloroform−0.1 M phosphoric acid; liquid−liquid partition into bicarbonate solution; solid-phase extraction onto a C18 column (Step-Bio, Bologna, Italy), and elution with ethyl acetate−methanol−acetic acid. OTA is then identified and quantified by an HPLC system equipped with a fluorescence detector, operated at an excitation wavelength of 333 nm and an emission wavelength of 460 nm.

The liquid chromatograph utilized was an HPLC model HP 1090 series II/L (Hewlett-Packard) connected with a fluorescence detector model HP 1046A (Hewlett-Packard) and with a HP 3396 series II (Hewlett-Packard) integrator.

Quantification of OTA was carried out by comparison to an external standard curve using OTA standard (Sigma, code O 1877, purity >98%). OTA detection limit was 0.1 μg/kg. Mean recovery from spiked sample ($n = 4$) at the 10 μg/kg level was 89% (RSD 5.6%).

Because the number of samples to be analyzed was large (162), a representative subsample (50 g) of each green coffee sample was extracted only once, but each final cleaned extract was analyzed twice by HPLC. To assess the method reproducibility, multiple analyses of some samples were performed; maximum and minimum relative standard deviations (RSD) were found to be respectively 17.06% and 4.90%. A sample was reanalyzed (extraction, cleanup, and HPLC) if there was good reason to question the accuracy of the result from the first analysis. On the basis of these results, we can affirm that the analytical procedure was satisfactory for the estimation of OTA in green coffee beans.

Statistical Analysis. The statistical significance of data was tested applying a non-parametric procedure (median test), in order to eliminate difficulties caused by heterogeneous data distribution. For statistical comparison of contamination rates, a χ^2 test (samples with >3 ppb vs positive with <3 ppb) was applied within the three geographical areas. In the construction of the box and whisker plots of samples, the outliers were selected adopting a coefficient of 1.5. Any values showing a distance higher than ±1.5 times the box height, respectively, from the inferior or the superior box values was selected as an outlier. The data were processed using the Statistica for Windows (Statsoft, Tulsa, OK) package.

RESULTS AND DISCUSSION

In Table 1 are the results obtained from the OTA analysis of 162 green coffee samples of various origins. From the analysis of 162 green coffee beans samples, 106 contained detectable amounts of OTA.

OTA was detected in the majority of the analyzed African samples (76/84) at levels ranging from 0.5 to 48 μg/kg, and the mean value of the overall samples was over 4 μg/kg. In particular, Zaire and Congo samples presented the highest mean as well as the maximum levels of toxin. However, because few samples were available from some locations, is not possible to conclude with confidence that there are significant differences of OTA contamination among coffees from the considered African sites. Among the green coffee samples from America, only 19 of 60 were positive with very low average content, and only two samples presenting more than 3 μg/kg of OTA. Eleven of the 18 Asian samples were positive for toxin with an average value of 1.06 μg/kg.

The frequency distribution patterns over different levels of OTA contamination are given in Figure 1a−c for the green coffee samples originating respectively from America, Africa, and Asia.

Figure 1a shows that over 65% of the samples were not contaminated and that samples with high levels (7−8 μg/kg) were very exceptional (only 2 Santos samples). The OTA content distribution of the samples originating from Africa is displayed in Figure 1b. Only about 10% of 84 African samples showed negative for OTA, and 50% of the samples showed OTA levels >3

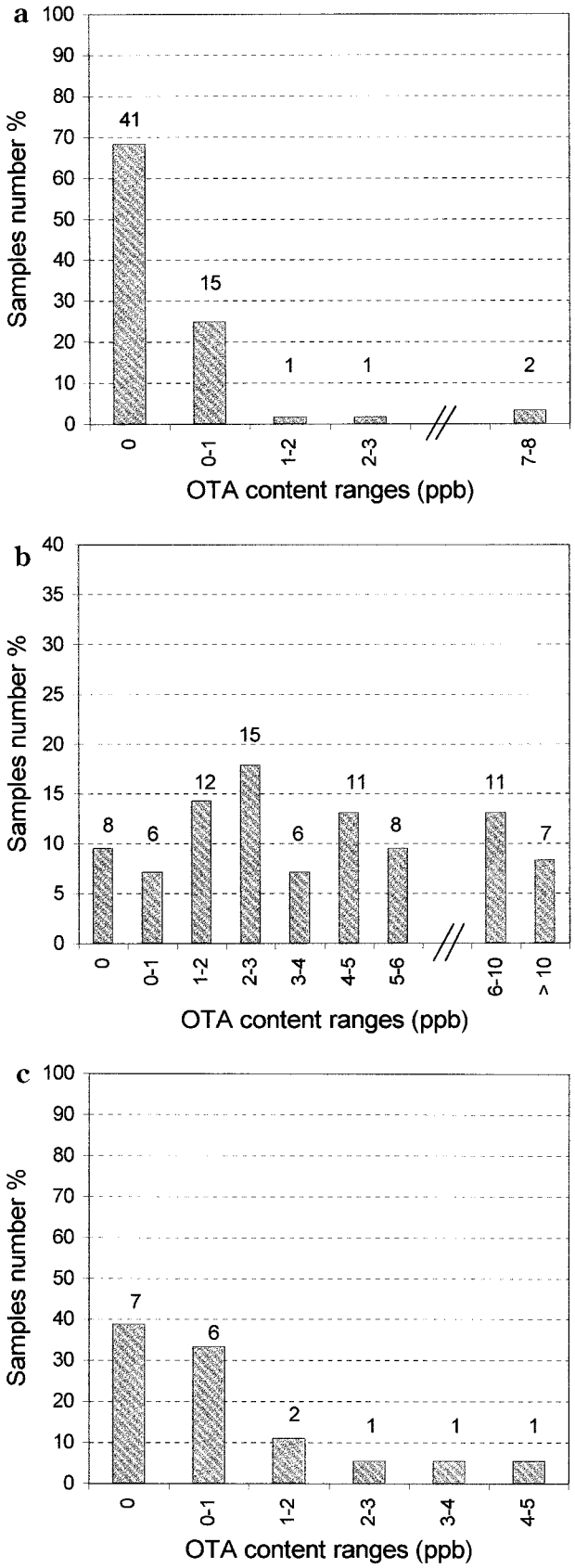

Figure 1. (a) OTA frequency distribution in American green coffee samples. Numbers reported on the histograms indicate the totality of samples set in each range. (b) OTA frequency distribution in African green coffee samples. Numbers reported on the histograms indicate the totality of samples set in each range. (c) OTA frequency distribution in Asian green coffee samples. Numbers reported on the histograms indicate the totality of samples set in each range.

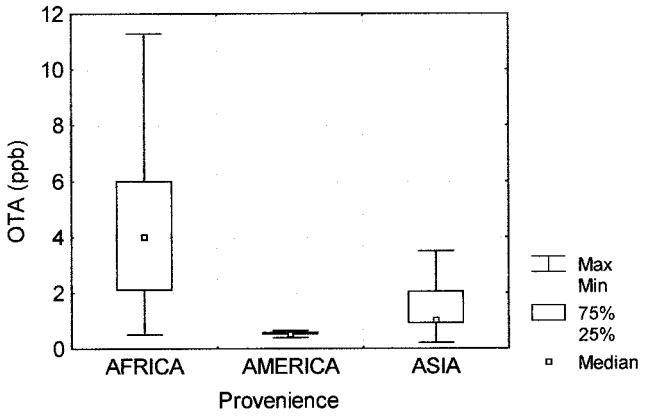

Figure 2. Box and whisker plots of positive green coffee samples OTA distribution as a function of their provenience.

Table 2. Results of Median Test of the Data Relative to OTA Levels in All Green Coffee Samples and Results of χ^2 Test for Comparison of Highly Contaminated Samples (Samples with >3 ppb vs Positive with <3 ppb) within the Three Geographical Areas

	median test			χ^2 test		
provenience	median (μg/kg)	$p < 0.05$	$p < 0.0005$	χ^2	df	$p <$
Africa	3.2	a	a	2086.11	32	0.0000
America	0.0	c	b	41.39	1	0.0000
Asia	0.9	b	b	204.50	1	0.0000

μg/kg. About 40% of the green coffee samples from Asia (Figure 1c) were negative for OTA, and only two samples showed OTA level over 3 μg/kg.

Figure 2 is the box plots showing the distribution (in terms of median, quartiles, and extreme values) of the positive samples in each geographical area. The visualization of outliers from each group was avoided in order to preserve a better understanding of the graph. It is noticeable that the OTA level of African samples shows a higher dispersion than the other two groups of green coffee samples. The box plots permit evidence that 75% of the African samples show OTA values considerably higher than the maximum extreme value of American samples and, at the same time, present toxin levels higher than that of over 75% of the Asian ones. Furthermore, the American samples showed OTA values lower than the 75% of the Asian ones.

The results of a median test performed on green coffee samples of different provenience are reported in Table 2. The comparison of the medians confirm that American samples had significantly lower OTA values than that of the other two groups of green coffees at a $p < 0.05$ confidence level. Asian samples showed OTA levels significantly lower than that of African ones at a $p < 0.05$ level. It is noticeable that the small number of Asian samples, together with asymmetry of data (Figure 2), could have somewhat influenced the difference between this and other two groups of samples at a $p < 0.0005$ confidence level.

A χ^2 test was carried out in order to compare highly contaminated coffee samples (samples >3 ppb vs positive with <3 ppb) within the three geographical areas (Table 2). On the basis of the results, it is possible to affirm that in any case the frequencies of samples contaminated at OTA levels >3 ppb are significantly different ($p < 0.0000$) from those contaminated at levels <3 ppb.

In conclusion, it is possible to assume that the data no doubt suggest that African samples were more highly

contaminated, in terms of frequency and level of OTA, than American samples. This fact is probably due both to the climatic conditions and to the crop processing condition of coffee in the different areas. It is noticeable that, in general, Arabica coffee is produced by a wet processing method and that Robusta and lower quality Arabica coffees are produced by a dry processing method.

As OTA occurrence seems to be region-dependent, further investigation could be performed on a much larger number of samples, over a number of years, to relate coffee toxicological quality to different provenience and to climatic, crop processing and storage conditions in order to orientate the choice of the coffee industry. For a great extent, this kind of approach could be interesting because it is not already clear if and how much of the OTA content could be reduced after the roasting process (Levi et al., 1974; Gallaz and Stalder, 1976; Cantafora et al., 1983; Tsubouchi et al., 1987; Micco et al., 1989; Studer-Rohr et al., 1994, 1995; Viani, 1996; Blanc et al., 1998).

LITERATURE CITED

Blanc, M.; Pittet, A.; Muñoz-Box, M.; Viani, R. Behavior of Ochratoxin A during Green Coffee Roasting and Soluble Coffee Manufacture. *J. Agric. Food Chem.* **1998**, *46*, 673−675.

Cantafora, A.; Grossi, M.; Miraglia, M.; Benelli, L. Determination of Ochratoxin A in Coffee Beans Using Reversed-Phase High Performence Liquid Chromatography. *Riv. Soc. Ital. Sci. Aliment.* **1983**, *12*, 103−108.

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

Illy, A.; Illy, E.; Macrae, R.; Petracco, M.; Sondahl, M. R.; Valussi, S.; Viani, R. The raw bean. In *Espresso Coffee. The Chemistry of Quality*; Illy A., Viani R., Eds.; Academic Press: San Diego, 1995.

ISO 4072-1982 (E). *Green coffee in bags−Sampling*; International Organization for Standardization: Geneva, 1982; pp 1−3.

Levi, C. P. Mycotoxins in Coffee. *J. Assoc. Off. Anal. Chem.* **1980**, *63*, 1282−1285.

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.

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.

Mislivec, P. B.; Bruce, V. R.; Gibson, R. Incidence of toxigenic and other moulds in green coffee beans. *J. Food Protect.* **1983**, *46* (11), 969−973.

Nesheim, S.; Stack, M. E.; Trucksess, M. W.; Eppley, R. M. Rapid Solvent-Efficient Method for Liquid Chromatographic Determination of Ochratoxin A in Corn, Barley, and Kidney: Collaborative Study. *J. AOAC Int.* **1992**, *75*, 3, 481−487.

Norton, D. M.; Toule, G. M.; Cooper, S. J.; Partington, S. R.; Chapman, W. B. The Surveillance of Mycotoxins in Human Food. In *Proceedings, Fourth Meeting on Mycotoxins in Animal Disease*; Pepin, G. A., Patterson, D. S. P., Gray, D. E., Eds.; Ministry of Agriculture, Fisheries and Food: Alnwick, Northumberland, 1982; pp 77−81.

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.

Stack, M. E.; Mislivec, P. B.; Denizel, T.; Gibson, R.; Pohland, A. E. Ochratoxins A and B, xanthomegnin, viomellein and vioxanthin production by isolates of *Aspergillus ochraceus* from green coffee beans. *J. Food Protect.* **1983**, *46* (11), 965−968.

Studer-Rohr, I.; Dietrich, D. R.; Schlatter, J.; Schlatter, C. Ochratoxin A in Coffee: New Evidence and Toxicology. *Lebensm.-Wiss. Technol.* **1994**, *27*, 435−441.

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

Tsubouchi, H.; Yamamoto, K.; Hisada, K.; Sakabe, Y. A Survey of Occurrence of Mycotoxins and Toxigenic Fungi in Imported Green Coffee Beans. *Proc. Jpn. Assoc. Mycotoxicol.* **1984**, *19*, 16−21.

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.

Viani, R. Fate of ochratoxin A (OTA) during processing of coffee. *Food Addit. Contam.* **1996**, *13*, 29−33.

Received for review July 16, 1999. Revised manuscript received April 26, 2000. Accepted May 13, 2000.

JF990783B