OCHRATOXIN A IN BRAZILIAN GREEN COFFEE[1]

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SUMMARY

Ochratoxin A is a nephrotoxic, teratogenic and imunotoxic compound produced by *Aspergillus* and *Penicillium* spp. It is a suspected carcinogen to humans and it is carcinogenic to rats. Recently it has drawn attention because it has been found in coffee and it has been the object of regulation by coffee importing countries. Brazil is the largest coffee producing country and its largest consumer. In order to conduct an initial assessment of the situation of the coffee produced in the country and offered to its population, one hundred and thirty two samples of Brazilian green coffee from 5 producing states (Minas Gerais, Paraná, São Paulo, Espírito Santo and Bahia) and destined for the home market, were collected at sales points at the cities of Londrina and Santos, Brazil, and analyzed for ochratoxin A. The toxin was isolated in immunoaffinity columns and quantified by HPLC with florescence detection. The limit of detection was 0.7ng/g and the average RSD for duplicates of the samples was 11%. Twenty seven samples were found contaminated with the toxin and the average concentration for the contaminated samples was 7.1ng/g ochratoxin A. Neither the total number of defects nor the number of specific defects according to the Brazilian coffee classification system (black, partly – black, sour, stinkers-black, stinkers-green, pod beans) showed any relation to the contamination of the samples with ochratoxin A.

Keywords: Mycotoxins; ochratoxin A; Brazilian coffee.

RESUMO

OCRATOXINA A EM CAFÉ VERDE BRASILEIRO. Ocratoxina A é um composto nefrotóxico, teratogênico e imunotóxico produzido por espécies de *Aspergillus* e *Penicillium*. Foi demonstrado ser carcinogênico para ratos e é possivelmente carcinogênico para humanos. Recentemente a toxina despertou atenção por ter sido encontrada em café e ter sido objeto de regulamentação por países importadores. O Brasil é o maior produtor de café no mundo e também seu maior consumidor. Para conduzir uma avaliação inicial da situação do café produzido no país e oferecido à sua população, cento e trinta e duas amostras de café verde brasileiro, provenientes de 5 Estados produtores (Minas Gerais, Paraná, São Paulo, Espírito Santo, e Bahia) e destinadas ao mercado nacional, foram coletadas em pontos de comercialização nas cidades de Londrina e Santos, Brasil, e analisadas para ocratoxina A. A toxina foi isolada em colunas de imunoafinidade e quantificada em cromatógrafo líquido de alta eficiência com detector de fluorescência. O limite de detecção foi 0,7ng/g e o coeficiente de variação médio entre duplicatas foi 11%. Vinte e sete amostras estavam contaminadas com a toxina e a concentração média para as amostras contaminadas foi 7,1ng/g de ocratoxina A. Nem o número total de defeitos e nem o número de cada defeito específico encontrado na classificação das amostras de acordo com o sistema brasileiro de classificação (preto, meio-preto, ardido, verde-preto, verdes, brocados, coco) mostrou ter qualquer relação com a contaminação presente nas amostras.

Palavras-chave: Micotoxinas, ocratoxina A, café brasileiro.

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1 – INTRODUCTION

Ochratoxin A is a toxic metabolite produced by *Aspergillus* and *Penicillium* spp. Among those *A. ochraceus* is pointed out as a main producer. Ochratoxin A is acutely toxic to all animals tested so far and this includes birds, mammals and fishes. It has also shown teratogenic and immunotoxic properties [2]. The kidneys are its main target organ. It is a potent carcinogenic agent to rats [19] and possibly carcinogenic to humans [8]. It has been suggested as the causative agent of the endemic human nephropathy affecting humans in the Balkans [14]. Nephropathies reported in Algeria and Tunisia have been correlated with the presence of the toxin in bodily fluids and in the food consumed by the population [4]. The natural occurrence of ochratoxin A in green coffee beans has been reported by several authors in concentrations ranging between 0.2 and 360ng/g [3,10,11, 12,13,16,17,18].

Recently ochratoxin A has drawn attention because it has been found in coffee and it has been the object of regulation by coffee importing countries [6,20]. Brazil is the largest coffee producing country and its largest consumer. In order to conduct an initial assessment of the situation of the coffee produced in the country and offered to its population, the present work examined samples of Brazilian green coffee from 5 producing states and destined for the home market collected at sales points in the cities of Londrina and Santos, Brazil. The incidence of ochratoxin A and the total number of defects or the number of specific defects (black, partly – black, sour, stinkers-black, stinkers-green, pod beans) were also examined for possible correlation.

2 – MATERIALS AND METHODS

2.1 – Sampling

One hundred and thirty two samples of Brazilian green coffee from 5 producing states (*Table 1)* and destined for the home market were collected at sales points in the cities of Londrina and Santos, Brazil. The samples were classified by the number of specific defects

(black, partly – black, sour, stinkers-black, stinkers-green, pod beans) according to the official Brazilian coffee characterization system [15]. About 300g of coffee grains were taken to the lab and dried for 16 hours at 50°C and then ground to 40mesh and kept in polyethylene flasks until analysis.

TABLE 1. Green coffee sample distribution according to state of origin in Brazil

State	Number of samples
Minas Gerais	35
Paraná	65
São Paulo	13
Espirito Santo	17
Bahia	2

2.2 – Ochratoxin A determination

The samples were extracted and submitted to cleanup as previously described by FURLANI & VALENTE SOARES [5] as follows: Ten grams of sample were extracted with 200mL 1% $NaHCO_3$ during 3 minutes in a blender at low speed. The extract was then filtered through a glass filter (Whatman GFB, USA) and an aliquot of 20mL was taken and added to 20 mL phosphate saline buffer (Sigma, USA). The resulting solution was applied to an immunoaffinity column (Ochraprep, Rhône-Diagnostics, UK). The column was washed with 20mL water and after that dry air was passed through the column. The toxin was eluted from the column with 1.5mL methanol: acetic acid (98+2) followed by 1.5mL water (0.5mL/min). The eluate was evaporated to dryness and dissolved with 1mL methanol: acetic acid (65+35). The immunoaffinity columns were used up to 3 times. After the first use the columns were reconditioned according to ZIMMERLI & DICK [21] and kept at 6-8°C until needed.

The extract eluted from the immunoaffinity column was injected into a high performance liquid chromatograph comprising: Waters pump, model 510; Rheodyne injector with 100mL loop; Hewlett Packard fluorescence detector, model 1046A; Waters integrator, model 740; Spherisob ODS-2 column, 5mm, 250 X 4.6mm. The mobile phase was methanol: 9% acetic acid (65:35) at 1.0mL/mim. The excitation and the emission wavelengths were 330nm and 470nm, respectively. Quantification was achieved by external standard. The Ochratoxin A standard was obtained from Sigma (USA). The standard solutions were prepared and calibrated according to AOAC INTERNATIONAL [1].

The analytical quality control included a recovery test in each series of samples analyzed. All immunoaffinity columns in the series were either new or were used the same number of times. The duplicates were analyzed on different days and the results were corrected for the recovery of the series.

3 – RESULTS AND DISCUSSION

Twenty seven samples were found contaminated with the toxin and the average concentration for the contaminated samples was 7.1ng/g ochratoxin A (*Table 2*). The limit of detection for ochratoxin A was 0.7ng/g ochratoxin A and the average RSD for duplicates of the samples was 11%. The multiple use of immunoaffinity columns resulted in acceptable recoveries for trace analysis at ppb level as well as the RSD for the duplicate results of the samples [7]. The results also showed that the specific brand of immunoaffinity columns used in the present work could be used for up to 3 times with green coffee samples (*Table 3*). A greater number of uses per column was not tried as they tend to clog after 3 uses with coffee samples. It should be noted that coffee is a specially hard sample to cleanup due to the presence of a great number of interfering compounds. On the other hand, the repeated use of the immunoaffinity columns during the cleanup step significantly lowered the costs of the analyses.

TABLE 2. Ochratoxin A in Brazilian green coffee

Samples		Ochratoxin A (ng/g) in contaminated samples	
Total	Contaminated	Range	Average
132	27	0.7 – 47.8	7.1

TABLE 3. Ochratoxin A recovery results for new and reused immunoaffinity columns

Level of use	Number of tests	Average recovery (%)	Recovery range (%)
1st use	9	80.4	69.7-94.4
2nd use	9	80.7	77.6-85.2
3rd use	2	80.3	77.3-83.3

The results of the present survey indicate that ochratoxin A contamination has low incidence and levels in Brazilian coffee when compared with data for coffee from other countries [6,20]. Of course, the ideal situation is the absence of such a contaminant in the commodity and research should be undertaken in order to pinpoint the critical steps in which the toxin is produced by the fungi as well as the ways to avoid such a contamination. A similar situation has been reported in the case of roast and ground coffees and of instant coffees offered to consumers within the country as the ochratoxin A levels found for were low [9].

TABLE 4. Defects (%) in the 130 green coffee samples analyzed for ochratoxin A.

	Range	Average
Black	0-12	0.58
Partly-black	0-1.40	0.26
Sour	0-12.3	2.18
Stinkers-black	0-3.90	0.41
Stinckers-green	0-24.0	4.6
Pod beans	0-1.0	0.05
Total	0-29.0	8.4

Neither the total number of defects nor the number of specific defects classified according to the Brazilian coffee classification system (black, partly – black, sour, stinkers-black, stinkers-green, pod beans) (*Table 4*) showed any relation to the contamination of the samples with ochratoxin A.

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