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Ochratoxin A in roasted coffee commercially sold in the city of Rio de Janeiro

Ocratoxina A em café torrado comercializado na cidade do Rio de Janeiro

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ABSTRACT

The objective of this study was to evaluate the incidence of ochratoxin A (OTA) in roasted and ground coffee commercially sold in Rio de Janeiro in 2002–2003 and 2012–2013. Samples were prepared using an immunoaffinity column, and OTA was quantified by HPLC using a fluorescence detector (LOD of 0.3 μg·kg^{-1} and LOQ of 0.7 μg·kg^{-1}). In total, 29 samples were collected in 2002–2003, with 12 samples containing levels below the LOD and 17 samples containing levels in the range of 0.3–5.7 μg·kg^{-1}. In the monitoring conducted in 2012–2013, 37 samples were evaluated, with only 02 samples having levels below LOD. The remaining 35 samples had levels in the range of 0.3–3.1 μg·kg^{-1}. OTA concentrations in all samples were lower than the maximum tolerable limit set by the Brazilian legislation (10 μg·kg^{-1}). However, it is relevant to stress the importance of OTA control in coffee as a product consumed daily by Brazilians.

KEYWORDS: Ochratoxin A; Coffee; High-performance Liquid Chromatography (HPLC); Sanitary Surveillance

RESUMO

O objetivo deste trabalho foi avaliar a incidência de ocratoxina A (OTA) em café torrado e moído comercializado no Rio de Janeiro em 2002/2003 e 2012/2013. As amostras foram preparadas usando uma coluna de imunoafinidade e a OTA foi quantificada por CLAE usando um detector de fluorescência (Limite de detecção, LOD = 0,3 μg.kg^{-1}, e Limite de quantificação, LOQ = 0,7 μg.kg^{-1}). Um total de 29 amostras foi coletado em 2002/2003, com 12 amostras contendo níveis abaixo do limite do LOD, a partir das quais 17 amostras tiveram níveis de concentração na faixa de 0,3 a 5,7 μg.kg^{-1}. No monitoramento realizado em 2012/2013 foram avaliadas 37 amostras, e apenas 02 amostras apresentando níveis abaixo do LOQ. A partir das quais 35 amostras tiveram níveis de concentração na faixa de 0,3 a 3,1 μg.kg^{-1}). As concentrações em todas as amostras foram menores que o limite máximo tolerável estabelecido pela legislação Brasileira (10 μg.kg^{-1}). Entretanto, é relevante ressaltar a importância do controle da OTA no café, como um produto consumido diariamente pelos Brasileiros.

PALAVRAS-CHAVE: Ocratoxina A; Café; Cromatografia Líquida de Alta Eficiência (CLAE); Vigilância Sanitária

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INTRODUCTION

Brazil is globally recognized as one of the main producers of coffee and needs to be seen as capable of producing high-quality coffee. The growth of microorganisms, particularly fungi, is a major cause of losses in the post-harvest period. Fungal growth is attributed to environmental conditions, mainly the high humidity levels prevalent in coffee-producing regions during the production cycle[1]. This growth may be followed by the production of mycotoxins[2], which are described as one of the first group of fungal metabolites toxic to animals[3]. At present, *Aspergillus carbonarius*, *A. niger*, and *A. ochraceus* has been identified as the species responsible for ochratoxin A (OTA) in Brazilian coffee beans[4]. Recently separated from the *A. ochraceus* taxon as a new species, *A. westerdijkiae* is also to able produce OTA[5]. OTA is classified by the International Agency for Research on Cancer (IARC) as part of the 2B group (i.e., possible human carcinogen) and has been shown to be nephrotoxic, teratogenic, immunosuppressive, and capable of inducing DNA damage in rodents[6]. Human exposure results from the ingestion of various foods; the main contributors of OTA to the diet are cereals and their derivatives (40%–50% of the total ingestion), with secondary sources including meat products, spices, beer, wine, teas, and fruit juices[7]. According to the literature, coffee significantly contributes to the total daily ingestion of this toxin[5]. Studies have shown that coffee is an adequate substrate for fungi that produce OTA[8,9]. Mycological analyses of cherry beans collected from trees have not revealed the presence of ochratoxigenic fungi, indicating that OTA contamination in green coffee is a post-harvesting phenomenon[10]. In Brazil, OTA has been detected in green coffee from various regions[11]. However, GOLLÜCKE *et al.*[12] observed lower concentrations of OTA in Brazilian coffee intended for export than in the green coffee commercially sold in Brazil. PRADO *et al.*[13] and LEONI *et al.*[14] detected OTA in both roasted and instant coffee. Because the OTA toxicology is associated with its presence in food, the Joint Expert Committee on Food Additives (JECFA) of the FAO/WHO has set the provisional tolerable limit of weekly ingestion at 100 ng/kg of body mass to ensure the prevention of nephrotoxic effects[7]. The maximum limits for OTA are stipulated in several countries. In Brazil, the RDC Nº 7 of February 2011 sets the maximum tolerable limit (MTL) at 10 $\mu g \cdot kg^{-1}$ for roasted and instant coffee[15]. Many studies are being conducted in the field of Hazard Analysis Critical Control Points (HACCP), providing important information on Good Agricultural Practices[16,17] that can contribute to more precise analytical results in the assessment of phytosanitary quality (e.g., contamination with OTA)[18], besides being useful in the monitoring of roasted and ground coffee, the end product of this production chain[13,14]. The objective of the present study was to assess the roasted ground coffee commercially sold in the city of Rio de Janeiro on the basis of monitoring work conducted in 2012–2013 for OTA contamination. The second objective was to compare these results with the results of monitoring work conducted between 2002 and 2003 at the National Institute for Health and Quality Control (INCQS).

MATERIALS & METHODS

Sampling

The samples were purchased in markets in the city of Rio de Janeiro between 2012 and 2013 and analyzed in the same period. In total, 37 samples were collected, representing 25 different brands. The total quantity of product contained in each package (500 g) was homogenized and divided into quadrants until an adequate quantity was obtained for analysis of two samples, each containing 25 g. The same procedure was used for samples of roasted coffee tested between 2002 and 2003, which included a total of 29 samples, purchased in markets, representing 11 different brands.

Standards and reagents

OTA standard (Sigma-Aldrich, St. Louis, MO, USA); acetonitrile (HPLC grade) and methanol (HPLC grade) (Tedia Company, Fairfield, NJ, USA); sodium bicarbonate ($NaHCO_3$), glacial acetic acid, and toluene (Merck, Darmstadt, Germany).

Extraction of OTA from coffee

Extraction of OTA from the coffee samples was performed according to PITTET *et al.*[19]. In total, 25 g of sample was mixed with 200 mL of methanol and a solution of 1% $NaHCO_3$ and then centrifuged for 15 min at 3,000 rpm and 20°C. The resulting extract was filtered through a glass filter (Whatman GFB, USA) under low vacuum, and a 5-mL aliquot of the filtrate was transferred to a volumetric flask. This volume was raised to 100 mL by the addition of phosphate-buffered saline (PBS) (Sigma, USA).

Immunoaffinity column clean-up

The resulting extracts were passed through an Ochratest® immunoaffinity column (Vicam, USA) at a flow rate of approximately 1 drop/s. The column was washed with 4 mL methanol, which was collected in a vial. The extract was evaporated to dryness under nitrogen stream and reconstituted with the mobile phase.

Method of analysis

The extraction process for determining OTA in roasted coffee used in the monitoring work performed in 2002–2003 and 2012–2013 was developed by PITTET *et al.*[19] and validated in-house[20]. Detection and quantification were performed by HPLC using a model LC 20AD Shimadzu chromatography apparatus equipped with an RF-20A fluorescence detector. The chromatographic conditions were as follows: Spherisorb Column, ODS, 5 µm, 250 × 4.6 mm, set at 25 ± 0.5°C. The fluorimetric excitation and emission wavelengths were set at 336 and 468 nm, respectively. The isocratic mobile phase was acetonitrile/acetic acid 2% in H_2O (1:1, v/v), with a flow rate of 1 $mL \cdot min^{-1}$. A five-point standard curve covering 0.15–2.15 ng OTA mL^{-1} was prepared ($r^2 > 0.9999$), and 20 µL of samples was injected in three trials. The typical retention time of OTA was approximately 9.5 min.

RESULTS AND DISCUSSION

The method used in this monitoring work was validated and the limit of detection (LOD) and limit of quantification (LOQ) of the chromatography procedure were determined at signal-noise ratios of 3:1 and 6:1, respectively. These were confirmed by spiking 0.3 and 0.7 μg·kg^{-1} OTA into three blank samples. Results are shown in Table 1.

These values are in agreement with those of the European Commission (2006)[17], from which the performance criteria for methods of OTA analysis are shown Table 2.

In 2002-2003, 29 samples of roasted coffee commercially sold in the city of Rio de Janeiro were analyzed. Two of these samples were from roasters in the city of Friburgo/RJ, in the highlands of the State of Rio de Janeiro, which used coffee from plantations located in the northwestern region of the state. The remaining 27 samples were from regions in the states of Sao Paulo and Minas Gerais. The range of OTA detected was 0.65-5.68 μg·kg^{-1}, with an average of 0.97 μg·kg^{-1}. The results of the contaminated samples were corrected by the values obtained in the validation (Table 1). These results are shown in Figure 01. In the monitoring of these 29 samples, contamination by OTA was not detected (ND) in 12 samples, i.e., < 0.3 μg·kg^{-1} (LOD).

Table 1. Recoveries and replicates (n = 3).

Spiking level (μg·kg^{-1})	Recovery (%)	RSDr (%)
0.3 (LOD)	58	14.2
0.7 (LOQ)	115	6.7
1.9	96	2.1
2.5	78	2.9
3.5	76	1.3
10.0	74	2.3

Table 2. Performance criteria for OTA.

Level μg.kg^{-1}	RSDr (%)	Recovery (%)
< 1	≤ 40	50 to 120
1-10	≤ 20	70 to 110

In the 2012-2013 monitoring of 37 samples of roasted coffee purchased in Rio de Janeiro supermarkets, testing for OTA revealed only two samples below LOD (< 0.3 μg·kg^{-1}). The range of OTA detected in the remaining samples was 0.3-3.1 μg·kg^{-1}, with an average of 0.81 μg·kg^{-1}. These results are shown in Figure 02.

Previous studies have assessed OTA in samples of roasted and ground coffee sold in Brazil. PRADO *et al.*[13] evaluated different brands of roasted ground coffee and instant coffee commercially sold in the city of Belo Horizonte/MG and found that OTA contamination for the roasted ground coffee was in the range of 0.9-5.9 μg·kg^{-1}, with an average of 1.7 μg·kg^{-1}. SABINO *et al.*[21] analyzed 82 samples of Brazilian instant coffee and found detectable levels of OTA (0.2-6.3 μg·kg^{-1}) in 81 (98.8%) of the samples. CORONEL *et al.*[22] evaluated roasted ground coffee purchased from supermarkets in Catalonia, Spain, and found OTA levels of 1.2-4.2 μg·kg^{-1}, with an average of 2.2 ± 0.8 μg·kg^{-1} (n = 72). The studies described above employed the same methodology for the identification and quantification of OTA as that used in the present study. We found that roasted coffee tested by several monitoring projects in Brazil contained OTA at concentrations from 0.2 to 6.3 μg·kg^{-1}, i.e., below MTL of 10 μg·kg^{-1} set by the RDC Nº 7 in February 2011[15]. Furthermore, the monitoring work of 2012-2013 revealed that roasted coffee samples purchased in Rio de Janeiro did not exceed MTL, with an average level of 0.81 μg·kg^{-1}. Only two samples did not contain detectable traces of OTA. This result differed from that of the 2002-2003 monitoring work, where although the average contamination was lower (0.96 μg·kg^{-1}), the percentage of contaminated samples was only 65.5%. From 2002-2003 to 2012-2013, the percentage of contaminated coffee samples increased.

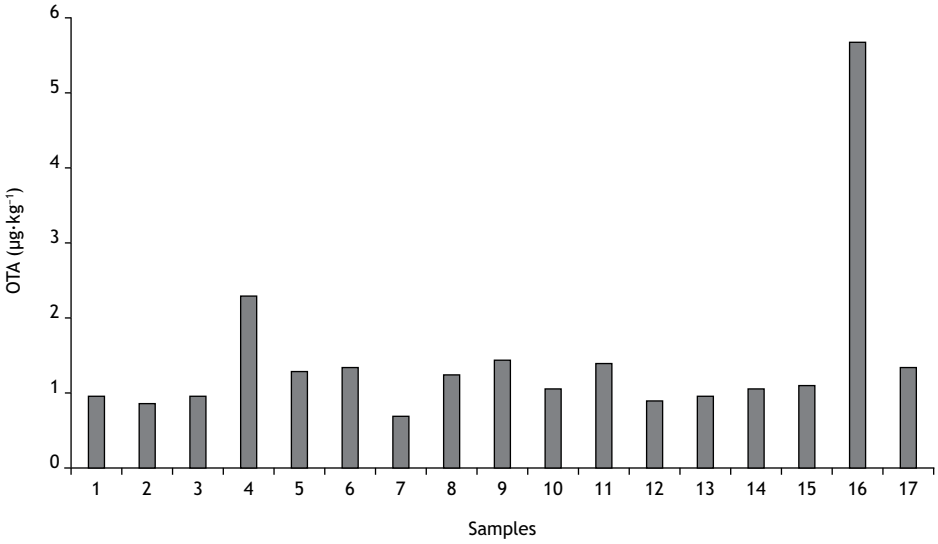

Figure 1. OTA contamination of coffee commercially sold in the city of Rio de Janeiro in 2002-2003, analyzed by INCQS/FIOCRUZ.

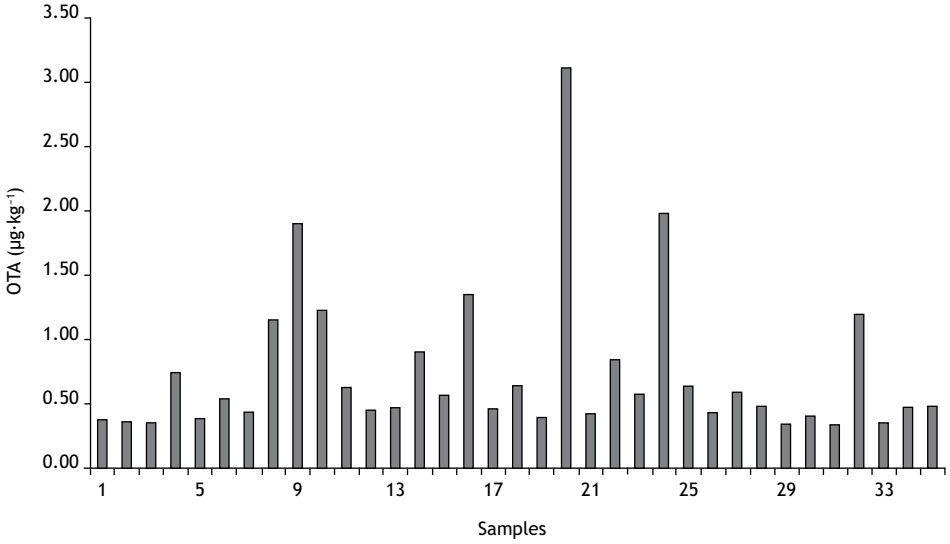

Figure 2. OTA contamination of coffee commercially sold in the city of Rio de Janeiro in 2012-2013, analyzed by UEZO/INCQS.

CONCLUSION

The Brazilian legislation[15] recommends that levels of mycotoxins in food be kept as low as reasonably possible through the use of best practices and technologies in production, handling, storage, processing, and packaging in order to prevent contaminated food from reaching domestic or foreign markets. It is imperative that good agricultural practices be applied by small- and mid-sized rural producers in the production chain. To ensure a product that meets consumer health and safety standards, producers and roasters should be alerted to avoid using low-quality grains in the blending of their coffee[23]. Our analysis of commercial coffee for OTA contamination conducted demonstrates the importance of effective regulation by governmental agencies because of the economic importance of this product in Brazil.

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