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Ochratoxigenic fungi and ochratoxin A in defective coffee beans

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ABSTRACT

Defective coffee beans may contain toxins such as ochratoxin A (OTA) and other compounds which affect health. In the present work two coffee producing regions were studied: Cerrado in Minas Gerais and Sorocabana in São Paulo State, with the objective of verifying the presence of ochratoxigenic fungi and OTA in the main defective coffee beans. Coffee samples were surface disinfected and plated directly onto Dichloran Glycerol Agar. Fungal species were isolated and identified. The coffee samples were analyzed for OTA and quantified by High Performance Liquid Chromatography (HPLC) equipment. *Aspergillus westerdijkiae* and *Aspergillus* section *Nigri* were found in both regions, while *Aspergillus carbonarius* only in Cerrado-MG, especially in defective coffee beans. The sour and black defective beans had the highest OTA concentration being 11.3 μg/kg and 25.7 μg/kg, respectively. In the defective green (immature) beans, although having the highest proportion (38%), the presence of ochratoxigenic fungi and OTA was low.

1. Introduction

It is estimated that more than two billion cups of coffee are drunk worldwide every day. Global consumption has almost doubled in the last 40 years and is forecast to reach more than nine million tonnes by 2019 (Fairtrade Foundation, 2012; ICO, 2013). Coffee is produced by more than 70 developing countries; in many of these, earnings from coffee exports are of vital importance to the country's balance of payments. Brazil has long been by far the world's largest coffee producer and exporter, growing an average of 2.5 million tonnes a year from 2007 to 2011 (Fairtrade Foundation, 2012). Apart from Brazil, the second largest coffee consuming country in the world, the major consumers are all in developed regions: USA, Europe and Japan. Coffee is the second biggest internationally traded commodity and thus plays a vital role in the balance of trade between developed and developing countries. Coffee is an important agent of social development and a great source of rural employment, providing a livelihood for some 125 million people around the world (Fairtrade Foundation, 2012; ICO, 2013).

The quality of the beans at the end of processing influences the price achieved when sold on the market and therefore, defects in coffee beans are undesired because they decrease the coffee quality. Coffee defects can be produced in the field, during harvest, processing, transport and storage; furthermore, they can be caused by physiological or genetic grain modification. They can be of intrinsic or extrinsic nature. Intrinsic nature is associated with beans irregular in visual appearance (black, sour, immature, black green, spongy beans and others), or associated with bean shape (shell, shell core, broken, insect damaged, pulper-nipped and others) (Illy & Viani, 2005; Toledo & Barbosa, 1998). On the other hand, defects of extrinsic nature are those represented by foreign matter, such as husks, sticks, stones and clods. All these defects depreciate the coffee price on the market. Furthermore, some of these defects alter the sensorial beverage characteristic considerably, adding off-taste such as fermented, woody, rotten fish, stinker and others (Illy & Viani, 2005; Toledo & Barbosa, 1998).

The differentiation between the defective and non-defective coffee beans was evaluated by several authors (Agresti, Franca, Oliveira, & Augusti, 2008; Toci & Farah, 2008), through volatile profile analyses. Toci and Farah (2008) found benzaldehyde and 2,3,5,6-tetramethypyrazine as potential markers for defective beans. However, no microbiological aspects were considered in these studies.

The permanence of coffee fruits in contact with the soil causes microorganism contamination and formation of black and sour defective beans. Some microorganisms can spoil the fruit, cause defects in the grain and produce toxins (Illy & Viani, 2005, Taniwaki, Pitt, Teixeira, & Iamanaka, 2003, Toci & Farah, 2008). A toxin which has been studied in coffee is ochratoxin A (OTA). This toxin is nephrotoxic, teratogenic and mutagenic in several animals (Van der Merwe, Steyn, Fourie, Scott, & Theron, 1965). It is produced principally by *Aspergillus ochraceus*, *Aspergillus westerdijkiae*, *Aspergillus niger* and *Aspergillus carbonarius* in coffee (Joosten, Goetz, Pittet, Schellenberg, & Bucheli, 2001; Morello et al., 2007; Noonim, Mahakarnchanakul, Nielsen, Frisvad, & Samson, 2008; Taniwaki et al., 2003; Urbano, Taniwaki, Leitão, & Vicentini, 2001). Other species isolated from coffee are *Aspergillus steynii*, *Aspergillus melleus* and *Aspergillus sclerotiorum* (Batista, Chalfoun, Prado, Schwan, & Wheals, 2003; Leong et al., 2007; Noonim et al., 2008) but these species are less common. OTA has been

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detected in coffee samples from all over the world (Urbano et al., 2001; Taniwaki et al., 2003; Pardo, Marin, Ramos, & Sanchis, 2004; Taniwaki, 2006; Noonim et al., 2008).

Based on the risk that OTA presents to human health, the European Union has developed regulations for the maximum levels of OTA permitted in a range of products including those of coffee. The maximum levels of OTA permitted in coffee are 5 μg/kg for both roasted coffee beans and ground coffee and 10 μg/kg for instant coffee (EC, 2005). In 2011, the Brazilian Sanitary Surveillance Agency (ANVISA) decided to set limits for ochratoxin A in roasted and soluble coffee. The set limits were 10 μg/kg for both types of coffee (ANVISA, 2011).

In 2001, the International Coffee Organization (ICO) and the Association of Coffee Producing Countries proposed the "Coffee Quality Improvement Programme" (ICO/ACPC no. 1/2001) which involved diverting lower grade coffee from the export market and also to alternative non-beverage uses (ICO, 2001). The coffee business for importing countries would benefit from this measure intended to improve quality. On the other hand, for producing countries such as Brazil, Colombia, Vietnam and more than 60 other countries, the consequence of this decision would not necessarily result in immediate gains. In fact, coffee growers would be adversely affected because the consequences would be the accumulation of defective coffee beans on the domestic market. Later on in 2004, the ICO adopted the Resolution 420 Coffee Quality-Improvement Programme Modifications, encouraging efforts to identify and put into practice cost-effective alternative uses for coffee which do not conform with the standards indicated in this document (ICO, 2004).

Due to the existence of defective coffee beans a more detailed study was required concerning OTA concentration, from a public health point of view, as well as alternative non-beverage products.

Further, no data were available about the mycobiota occurring in the different defective coffee beans, which would give information on the source of potential toxins that could be found in coffee. Therefore, the aims of this research were: (i) to investigate the presence of ochratoxigenic fungi in the main defective beans and their potential to produce ochratoxin A in culture media; (ii) to check the presence of ochratoxin A (OTA) in defective and normal coffee beans, and (iii) to identify the mycobiota in the main defective coffee beans such as, green (immature), black, black green and sour compared to normal beans.

2. Material and methods

2.1. Coffee samples

Two bags of 60 kg of defective coffee beans *Coffea arabica* (arabica) considered as residual coffee by a sorting machine were analyzed. These coffee bags came from two different Brazilian coffee producing regions: a) Cerrado Mineiro, in the Western part of Minas Gerais State, a temperate, dry region (average of 19.1 °C and 14.8 mm/month rainfall), with high altitude (>1100 m), and producing good quality beverage; and b) Sorocabana in South Western São Paulo State, a relatively cold, rainy region (average of 17.7 °C and 64.9 mm/month rainfall), also of low altitude (<800 m), and generally producing low quality beverage.

2.2. Separation of coffee defects

Two samples from the 60 kg defective coffee bags were manually separated by visual aspects into: normal, green (immature), black, black green and sour beans. The appearance and characteristics of these beans are shown in Fig. 1. These separated coffee beans were used for water activity, mycological and ochratoxin A determinations.

For calculation of the percentage of defects in each bag, another sample of 2 kg was collected in a different part of the bag and, from that, a subsample of 200 g was taken. The calculation of the defects was based on the 200 g. Each defective bean was separated manually as previously mentioned.

2.3. Water activity

Water activity was determined by using an AquaLab Series 3TE instrument (Decagon, USA) at 25 ± 0.1 °C, in triplicate.

2.4. Mycological analysis

Samples of coffee beans were surface disinfected with 0.4% chlorine solution for 1 min (Pitt & Hocking, 2009), and then a total of 33 or 50 beans was plated directly (10 to 11 particles per plate) onto Dichloran 18% Glycerol Agar (Hocking & Pitt, 1980). The plates were incubated at 25 °C for 5 days. After incubation, the plates were examined for colony growth visually and with the aid of a stereomicroscope. All the fungal species were first isolated in Petri plates containing Czapek Yeast Extract Agar (CYA) to be later identified by specific protocols for each genus.

2.5. Identification of fungi

The isolated *Aspergillus* spp. and *Penicillium* spp. were grown in CYA and Malt Extract Agar (MEA). The genus *Penicillium* was identified according to Pitt (2000) and Samson, Hoekstra, Thrane, Frisvad, and Andersen (2010), and the identification of genera *Aspergillus* and *Eurotium* was performed according to Klich and Pitt (1988), Samson et al. (2010) and Frisvad, Frank, Houbraken, Kuijpers, and Samson (2004). *Fusarium* species were identified according to Nelson, Toussoun, and Marasas (1983) and Leslie and Summerell (2006). The other fungi were identified according to descriptions of Pitt and Hocking (2009) and Samson et al. (2010), supplemented with other sources when necessary.

Isolated *Aspergillus* spp. were inoculated in 3 points in the plates of CYA and MEA and incubated for 7 days at 25 °C. Its teleomorphic state *Eurotium* spp. was cultivated in Czapek Yeast Extract Agar with 20% Sucrose (CY20S) for 14 days at 25 °C. The numbers of isolates identified as *A. westerdijkiae* or closely related species in *Aspergillus* section *Circumdati*, *A. carbonarius*, and other *Aspergillus* section *Nigri* were counted for each sample, and the infection percentage of the coffee beans calculated. The species of *Penicillium* were grown by following the conditions above and then inoculated in CYA at 5 °C and 35 °C. After the cultivation period, the diameters of the colonies were measured and the macro- and microscopic features observed in each culture medium were used for species identification.

The species of *Fusarium* were grown monosporically in Carnation Leaf-Piece Agar (CLA) plates and Potato Dextrose Agar (PDA) slants for 10–14 days and subsequently were identified following Nelson et al. (1983) and Leslie and Summerell (2006).

2.6. Test for OTA production by isolated fungi

The isolates identified as *A. westerdijkiae* or closely related species of *A.* section *Circumdati*, *A.* section *Nigri* and *A. carbonarius* were grown on Yeast Extract 15% Sucrose Agar at 25 °C for 7 days and evaluated for the production of OTA by the agar plug technique, which tests small samples from Petri dishes by thin layer chromatography (TLC) (Filtenborg, Frisvad, & Svendensen, 1983). The TLC plates were developed in toluene/ethyl acetate/formic acid (5:4:1) and visualized under UV light at 365 nm. An OTA standard (Sigma Chemical Co., St Louis, USA) was used for comparison.

2.7. Determination of OTA in coffee

Ochratoxin A from coffee beans was analyzed according to Vargas, Santos, and Pittet (2005) with in house validation. A 25 g aliquot of coffee samples was extracted with 200 mL of a mixture of methanol:

a)  Normal bean: without any physic or chemical changes.

b)  Green bean: unripe bean with greenish silver-skin colour. From immature fruit harvest.

c)  Black bean: endosperm of the bean black. From delayed harvest of coffee, long contact with ground and other causes.

d)  Black-green bean: unripe bean with bright black-green silver-skin color. Immature fruits harvested and dried at temperature higher than 30ºC.

e)  Sour bean (brown bean): endosperm of the bean with a range of brown and red colors. From fruits harvested immature or overripe, long contact with ground and other causes.

Fig. 1. Types of coffee beans studied: a) Normal, b) Green, c) Black; d) Black green and d) Sour.

3% aqueous sodium bicarbonate (50:50). Extracts were filtered, and 4 mL was taken and diluted with 100 mL of phosphate buffered saline and applied to an immunoaffinity column (Vicam L.P., Watertown, MA) containing monoclonal antibodies specific for OTA. After washing with distilled water, OTA was eluted with 4 mL of HPLC grade methanol. This extract was dried under N2 and resuspended with 300 μL of mobile phrase and quantified by reverse-phase HPLC with fluorescence detection. A volume of 20 μL was injected through a SIL-10ADVP auto injector (Shimadzu Corporation, Japan). The mobile phase was methanol: acetronitrile:water:acetic acid [35:35:29:10, (v/v/v/v)], with an isocratic flow rate of 0.8 mL/min using a SLC-10AVP pump (Shimadzu Corporation, Japan). The equipment used was a Shimadzu LC-10VP system (Shimadzu Corporation, Japan) with a fluorescence detector RF-10AXL (Shimadzu Corporation, Japan) set at 333 nm excitation wavelength and 477 nm emission wavelength. Chromatographic separation was achieved using a Supelcosil™ LC-18 column (5 μm, 250 × 4.6 mm, Supelco, Bellefonte, PA) equipped with an ODS Hypersil pre-column (5 μm, 25 × 4.6 mm) placed into a CTO-10ADVP column oven (Shimadzu Corporation, Japan) at 40 °C. The method to analyze OTA in raw coffee was already validated in previous publications (Gollücke, Taniwaki, & Tavares, 2004, Taniwaki et al., 2003). For recovery studies, coffee samples were spiked with OTA standard (Sigma, USA) at three levels 4.8, 8 and 80 μg/kg. The detection and quantification limits were obtained by analyzing 10 repetitions of raw coffee contaminated with lower levels of ochratoxin A (<0.2 μg/kg).

3. Results

3.1. Defective beans

Table 1 shows the percentage of each type of defective coffee bean: green (immature), black, black green and sour in samples collected from Cerrado-MG and Sorocabana-SP regions. The green defect which refers to immature beans, was the most common, reaching 30.5% and 38% in Cerrado-MG and Sorocabana-SP, respectively. Other common defects were the black and black green appearing in 10.7% and 16.2% in Cerrado-MG and Sorocabana-SP, respectively.

3.2. Mycobiota of normal and defective coffee beans

Fig. 2 shows the coffee beans infected with ochratoxigenic fungi on DG18. The percentage of defective coffee beans infected with fungi,

Table 1
Percentage of defective coffee beans samples from Cerrado-MG and Sorocabana-SP.

Type of defect	Cerrado-MG (%)	Sorocabana-SP (%)
Green	30.5	38
Black	10.7	4.9
Black green	5.1	16.2
Sour	9.8	3.3
Other undefined defects	43.9	37.6
Total	100	100

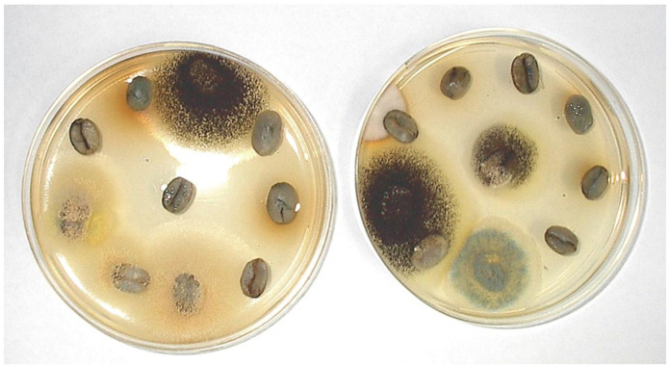

Fig. 2. Coffee beans infected with fungi on DG18.

referring to Cerrado-MG and Sorocabana-SP regions, and the water activity are shown in Table 2. The water activity in Cerrado-MG of normal beans was lower than that of defective beans, while in Sorocabana-SP, the defective beans had lower water activity. *A. westerdijkiae* and *Aspergillus* section *Nigri* were found in both regions, while *A. carbonarius* only in Cerrado-MG, especially in defective coffee beans. Compared to normal beans and defective coffee beans, infection with ochratoxigenic fungi in normal beans was also found being *A.* section *Nigri* (2%) and *A. westerdijkiae* (12%) in Cerrado-MG and Sorocabana-SP, respectively.

All defective beans (green, black, black green and sour) from Cerrado-MG (Table 2) were infected with *A. carbonarius*, *A.* section *Nigri*, *A. westerdijkiae* or *A.* section *Circumdati*. Only in sour beans was *A. flavus* found (which can also produce aflatoxin type B), but at a low incidence (2%). Other species found in defective coffee beans from Cerrado-MG were: *Eurotium chevalieri*, *Eurotium rubrum*, *Fusarium oxysporum*, *Fusarium lateritium*, *Fusarium* spp. and dematiaceous fungi. On the other hand, all defective coffee beans from Sorocabana-SP (Table 2) showed infection with *A. westerdijkiae* at different percentages (3 to 33%), with black beans being the highest. *Aspergillus* section *Nigri* were found only in sour beans at a low level (6%). Other species found in Sorocabana-SP were: *E. chevalieri*, *Eurotium repens*, *F. oxysporum*, *F. lateritium*, *Penicillium brevicompactum* and dematiaceous fungi. The black and sour defective beans had the highest infection by ochratoxigenic species in both regions.

3.3. Potential for ochratoxin A production by isolates

The potential for ochratoxin A production by *A. carbonarius*, *A.* section *Nigri*, *A. westerdijkiae* and *A.* section *Circumdati* isolates is shown in Table 3. All isolates of *A. carbonarius* and *A. westerdijkiae* produced OTA, while 0 to 23% of *A.* section *Nigri* and 54 to 100 of *A.* section *Circumdati* produced OTA. Considering the potential for OTA production in coffee, black and sour defective beans had the highest potential.

3.4. Presence of OTA in coffee

Using the methodology described in Section 2.7, recoveries of ochratoxin A were 86.5, 78 and 81% for spiking levels of 4.8, 8.0 and 80.0 μg/kg, respectively. The detection and quantification limits of the method were 0.2 and 0.6 μg/kg, respectively. OTA levels were expressed as μg/kg of coffee on a wet basis. Fig. 3 shows the chromatogram of coffee beans naturally contaminated with 11.3 μg/kg of OTA.

The levels of OTA in normal and defective beans in Cerrado-MG and Sorocabana-SP regions are shown in Table 4. The sour and black defective beans from Cerrado-MG and Sorocabana-SP regions showed the highest levels of OTA, being 11.3 μg/kg and 25.7 μg/kg, respectively.

4. Discussion

The ochratoxigenic species found in this study were: *A. carbonarius*, *Aspergillus* section *Nigri*, *A. westerdijkiae* and other *A.* section *Circumdati*. Although all isolates of *A. carbonarius* produced OTA, this species was found at a low percentage and only in the Cerrado-MG region. This species is common in grapes and in robusta coffee but is not common in Brazil, unlike in Thailand, where this species is commonly isolated (Leong et al., 2006; Noonim et al., 2008; Taniwaki et al.,

Table 2
Percentage of fungal infection and water activity of normal and defective coffee beans (green, black, black green and sour), from Cerrado-MG and Sorocabana-SP.

Coffee region	Cerrado-MG (% fungal infection)					Sorocabana-SP (% fungal infection)				
Mean a_w (range)[a]	0.556 (0.554–0.559)	0.619 (0.619–0.621)	0.620 (0.619–0.623)	0.617 (0.616–0.619)	0.613 (0.610–0.618)	0.662 (0.628–0.697)	0.592 (0.584–0.599)	0.590 (0.580–0.601)	0.589 (0.583–0.600)	0.567 (0.564–0.572)
Fungal species	Normal	Green	Black	Black green	Sour	Normal	Green	Black	Black green	Sour
Aspergillus carbonarius	–	6	8	–	6	–	–	–	–	–
Other *Aspergillus* section *Nigri*	2	28	26	38	32	–	–	–	–	6
Aspergillus flavus	–	–	–	–	2	–	–	–	–	–
Aspergillus westerdijkiae	–	6	–	–	2	12	3	33	6	9
Other *Aspergillus* section *Circumdati*	–	4	26	22	26	–	–	–	–	–
Aspergillus sydowii	2	–	–	–	–	–	–	–	–	–
Alternaria sp.	–	–	–	–	–	3	–	–	–	–
Cladosporium cladosporioides	–	–	2	–	–	–	–	–	–	–
Eurotium chevalieri	–	14	8	10	16	–	3	–	–	–
Eurotium repens	–	–	–	–	–	3	3	–	–	3
Eurotium rubrum	2	6	–	4	–	–	–	–	–	–
Eurotium spp.	–	–	–	–	2	–	–	3	–	–
Fusarium equiseti	–	–	–	–	–	–	–	–	–	3
Fusarium lateritium	2	–	2	–	–	3	–	12	–	3
Fusarium oxysporum	–	–	2	–	–	–	–	–	–	–
Fusarium semitectum	–	–	–	–	–	3	–	3	–	–
Fusarium spp.	2	–	10	2	2	–	–	–	–	–
Mucor sp.	–	2	–	–	–	–	–	–	–	–
Nigrospora sphaerica	–	2	–	–	–	–	–	–	–	–
Penicillium brevicompactum	8	–	–	–	–	–	–	15	–	–
Penicillium spp.	–	–	–	–	–	–	3	–	–	–
Dematiaceous fungi	–	2	4	–	4	9		18	–	3

[a] Mean of three repetitions.

Table 3
Number of isolates and percentage (%) of ochratoxin A positive isolates from Cerrado-MG and Sorocabana-SP.

Fungi	Normal n (%)	Green n (/%)	Black n (%)	Black green n (%)	Sour n (%)
Cerrado-MG					
Aspergillus carbonarius	–	3 (100)	4 (100)	–	3 (100)
Aspergillus section *Nigri*	1 (0)	14 (14)	13 (23)	19 (5)	16 (12)
Aspergillus westerdijkiae	–	3 (100)	–	–	1 (100)
Aspergillus section *Circumdati*	–	2 (100)	13 (54)	11 (80)	13 (54)
Sorocabana-SP					
Aspergillus section *Nigri*	–	–	–	–	2 (0)
Aspergillus westerdijkiae	4 (50)	1 (100)	11 (100)	2 (100)	3 (100)

Table 4
Levels of ochratoxin A (μg/kg) of normal and defective coffee beans (green, black, black green and sour), in Cerrado-MG and Sorocabana-SP.

Type of defect	Cerrado-MG (μg/kg)	Sorocabana-SP (μg/kg)[a]
Normal	ND[b]	1.4
Green	ND	0.3
Black	0.5	25.7
Black green	0.4	0.3
Sour	11.3	0.3

[a] Average of 3 repetitions.
[b] Not detected (limit of detection 0.2 μg/kg).

2003). *A. westerdijkiae* and *A.* section *Circumdati* were found at a higher percentage in both regions and these species may be the main source of OTA in these defective beans, since most of *A.* section *Nigri* did not produce OTA. *A. westerdijkiae* was described by Frisvad et al. (2004) as belonging to *Aspergillus* section *Circumdati*, and it has been recognized as the main OTA producer in coffee by several authors (Frisvad et al., 2004; Morello et al., 2007; Noonim et al., 2008; Taniwaki, 2006). According to the data presented by Silva, Batista, Abreu, Dias, and Schwan (2008), species of potentially OTA-producing fungi become prevalent in the last days of 22 days of fermentation and drying through the 'dry' processing of coffee beans.

Vilela et al. (2010) using a polyphasic approach for characterizing the mycobiota of semi-dried processed coffee found *Aspergillus chevalieri*, *Cylindrocarpon* sp., *E. chevalieri*, *Fusariella* sp., *Fusarium solani* and *Phoma* sp. as the least common fungi, and *Aspergillus tubingensis*, *Aspergillus versicolor*, *Cladosporium cladosporioides*, *Aspergillus* sp. and *Penicillium decumbens* as the most common species. Some of the filamentous fungal species were associated with good quality coffee, such as *C. cladosporioides*, while others depreciated the quality, including *Fusarium* and *Penicillium* (Vilela et al., 2010). Among the mycotoxigenic species, *A. ochraceus* was detected. In the present study, most of the coffee samples from both origins showed the presence of xerophilic *Eurotium* species such as *E. chevalieri*, *E. repens* and *E. rubrum*. This may be due to the low water activity found in these samples (from 0.554 to 0.697).

The green (immature) was the most common defective bean (38%) in coffee residual samples in both regions of Cerrado-MG and Sorocabana-SP. The presence of ochratoxigenic fungi and OTA in this type of defect was low. This would be expected since the origin of these defective beans is when harvesting unripe cherries. These beans usually do not suffer attack by fungi but cause an undesirable flavor in coffee beverage, described as an increased bitterness (ISO, 1993) and it is characterized as metallic and astringent (Illy & Viani, 2005). The black green beans also had a low level of OTA in both regions, similar to green (immature) defective beans, but they were also dried or exposed to an excessive temperature. The black beans had the highest OTA (11.3 μg/kg) concentration in the Cerrado-MG region, and the reason may be due to overripe cherries on the tree or those fallen on the ground or during processing. The high infection by ochratoxigenic fungi and OTA formation indicate that the fungi had the opportunity to grow and produce the toxin in the coffee. According to Illy and Viani (2005) 10% of black beans cause noticeable harm to the beverage by producing a harsh and ashy flavor. The sour beans had the highest OTA concentration in the Sorocabana-SP region, due to deterioration from excessive fermentation, causing a sour taste in the beverage.

5. Conclusion

The present work helps to find alternatives for defective coffee beans. Green (immature) defective beans, which were found the most, could have more aggregate value such as the production of cosmetics since the OTA level was very low. The black and sour defective beans showed the highest percentage of ochratoxigenic fungi and OTA, these beans could be diverted from beverage, using alternatively as fuel.

In conclusion, the sour and black defective beans had the highest OTA concentration. In the defective green (immature) beans, the presence of toxigenic fungi and OTA was low. The reduction of defective beans in coffee lowers the level of infection by ochratoxigenic fungi, reduces ochratoxin A content and improves considerably beverage quality.

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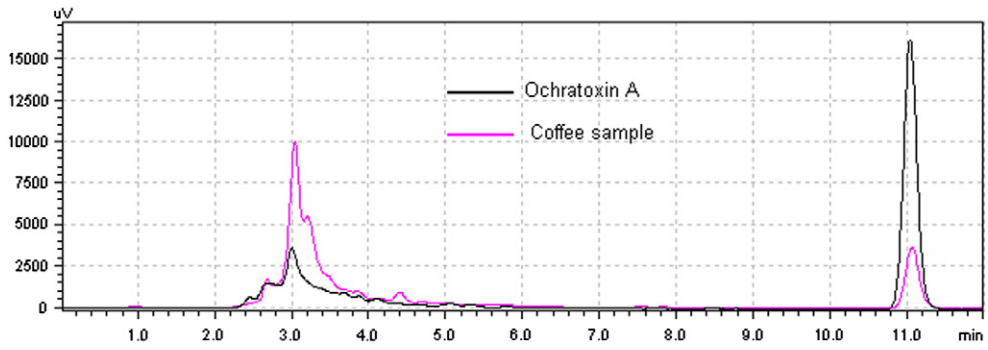

Fig. 3. Coffee sample naturally contaminated with ochratoxin A and OTA standard.

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