Influence of Coffee Processing and Defects on the Incidence and Occurrence of Ochratoxin A

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SUMMARY

762 coffee samples (1 kg) – most of dried processed arabica coffee from several stages of pre and post harvest – were collected from different regions of Brazil according to a sample history questionnaire. Among them 60 samples (16 kg) were classified and sorted by defects, according to Brazilian Classification, in 13-17 types of defects: black, sour, insect damage beans, malformed, shell, immature, bean with fox silver skin, pulp nipped bean, broken, parchment, sticks, among others. All 762 samples and fractions of defects (446 subsamples) in the coffee samples were analysed for OTA and the influence and impact of coffee processing and the presence of defects in the OTA contamination were determined.

INTRODUCTION

Ochratoxin A (OTA) a naturally occurring mycotoxin in coffee and other products is produced by the genera *Aspergillus* and *Penicillium*. It is considered a renal carcinogenic to animals and possibly to humans (IARC, 1993) and its presence in food has been a concern in many countries which have been laid down regulation and guidelines (FAO, 1997; CE, 2002a; CE, 2002b). During cultivation, processing and hulling, coffee is subject to a hazard i.e. ochratoxin A contamination. The implementation of good agricultural practice may minimize contamination; therefore, the identification of critical points is fundamental for the implementation of the HACCP system. Many factors have been reported in literature as critical for the production of a good quality and safe coffee such as cultivation practices, harvest, wagging, separation or washing, drying: terrace management and type of terrace, storage, hulling and transportation (PAS, 2004). The presence of defects in coffee is an indicative of its quality given by a type number (Brasil, 2003) and will determine the price and its acceptability in the market (Mattielo, 1991). In this study, we have characterized some aspects of coffee quality (defects), cultivation and processing that contribute to the occurrence of ochratoxin A in coffee, specially those related to dry processing such as the harvest and post harvest reflected herein as floats, natural cherry; husked cherry; mixture of unripe/natural cherry; mixture of unripe/ripe and floating (*"café da roça"*) and sweeping coffee beans.

EXPERIMENTAL

Part 1

762 coffee samples (1 kg), most of dried processed arabica coffee, were collected from different regions of Brazil according to a sample history questionnaire what allowed the characterization of the farm infrastructure and facilities, as well as the characterization of approximately 12 aspects of the agricultural practices involved in the pre-harvest and pos-harvest of the sample such as shading, fertilization, irrigation, coffee variety, degree of maturation, type of harvest (mechanical and manual), wagging, contact with soil, separation and washing, drying process (terrace, dryers). The effect (independent) of each aspect of the agricultural practice in the occurrence of OTA contamination was determined using the Qui-square (χ^2) and the exact Fisher test. For the evaluation of the difference between the types of coffee the t student test was applied. The influence of the occurrence of OTA in coffee was done using logistic regression based on categorical data with probability of significance below 25% selected through the univariate analysis. All results were considered significant for a probability smaller than 5% ($p < 0.05$), having, therefore, at least 95% confidence level at the conclusions presented.

Part 2

Sixty (60) green coffee samples were collected according to a sampling protocol designed for green coffee (Vargas et al., 2004a, 2004b). The 16 kg sample were homogenised and divided in 03 samples (~5 kg). One of the 5 kg samples was split in 02 samples of 1 kg and 4 kg sample named original and composed sample, respectively. The 04 kg samples were decomposed (hand picked) in defect free (picked-up) and defect subsamples. The defects were sorted out and classified according to the Brazilian Classification System and ISO 10470 in 13-17 types of defects taking in consideration the most severe defect giving a total of 446 defect subsamples: As an example a sour/insect damaged bean was classified as a sour bean. The contribution of the mass of the defect and of the picked-up sample, as well as their contamination to the overall OTA contamination of the composed sample (4 kg) was determined by using the following equations:

$$\text{OTA}_{\text{Composed}} = \frac{\sum_{i=1}^{n} Mass_i \times OTA_i}{\sum_{i=1}^{n} Mass_i}$$

where: $Mass_i$ → Mass of the defect sample i
OTA_i → level of contamination of the defect i

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The **t** Student test was used to evaluate the difference between the OTA contamination levels in the original and composed sample, and in defect free subsamples and defects. The relationship between OTA contamination, quantity, and mass of defects was determined using Pearson's correlation (r). The frequency of a given defect in composed samples with contamination> 5 ng/g was determined by using Fisher test. All results were considered significant for a probability of $p < 0.05$. The test non-parametric "Kappa" was used to estimate the degree of concordance between the number (%) of samples with contamination i.e. ≤5 ng/g and >5 ng/g due to mass (1 kg and 4 kg) and presence of defects.

All samples and defects (part 1 and part 2) were prepared by grinding down the coffee beans through 0.5-1.0 mm screens and thoroughly homogenised. Samples were analysed by immunoaffinity column with liquid chromatography (Vargas et al., 2003).

RESULTS AND DISCUSSION

Part 1

The distribution of the 762 samples classified according to type of coffee was husked cherry (28.9%), mixture (20.5%), sweeping coffee (varrição) (20.1%), float (15.9%), unripe/natural cherry (8.0%), and natural cherry (6.7%). The **a**nalysis of OTA in the 762 samples revealed a frequency of 83.6% of samples with contamination up to 5ng/g with 48.4% of the sample free of OTA (nd = 0.06 ng/g, LD = 0.12 ng/g), and 16.4% of samples with OTA contamination ≥ 5ng/g (Figure 1).

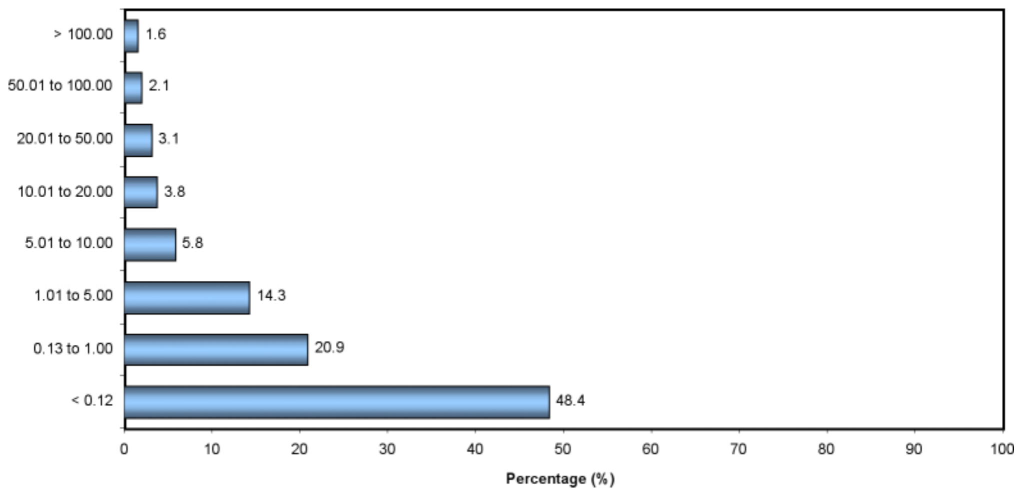

Figure 1. Distribution of samples per range of OTA contamination.

There were significant differences between the contamination of sweeping coffee (*varrição*) and other types of coffee. It was observed that coffee from sweeping was highly likely to increase the contamination of the whole samples significantly, in the extent of 41.8% of the samples with OTA contents > 5 ng/g (16.8%) (Table 1 and 2). Standing as second major contributor towards the presence of OTA, float type occurred in 20.6% of the samples with OTA >5 ng/g. The two types (sweeping and float) were originated in the plantation site, occasioned by the drying of the fruit on the trees and from the ones fallen down on the soil. It is remarkable to notice that even coffees from sweeping show a certain percentage of samples free of OTA, indicating the existence of pre-conditions for the occurrence of OTA. In this respect the hypothesis is that the degree of humidity of the soil, the time of contact with the soil and the extent of damage suffered by this fallen-down coffee when its permanence on the tree, are pre-conditions for major or minor occurrence of OTA. The occurrence of coffee berry borer on the coffee, in its various stages of development and the occurrence of damages, both physical and phyto-pathological to the fruit when still on the tree, thus causing ruptures for precocious contamination by fungi and production of OTA. Still on the Table 1 and 2 one can observe the development of OTA in the mixture green/natural cherry (17.6%), which was attributable to improper handling of these coffees in the drying terrace. The complete mixture (T5) of unripe and ripe cherry, raisin/dry (floats) on the tree, as it is customarily done in most of Brazilian harvest has presented a sampling contribution of 14.5% > 5 ng/g. Such contamination is expected to have been partly originated from raisin/dry coffees from the tree, already with a high fungus invasion, and partly has been developed at the drying terrace, due to either deficiency or inadequacy of the drying process. Lastly, it was observed that the presence of OTA in husked cherry coffee was practically not existing (1.1%). That stands for the absence of toxin in cherry coffee, i. e., in the mature and healthy form, as the coffee should be harvested, as well as the absence of contamination of the product in the post-harvest process, indicating that the drying process has been well conducted.

Table 1. Descriptive analysis OTA contamination per group of coffee: sweeping coffee x others

Types of coffee	Descriptive Analysis					
	Minimum	**Maximum**	**Median**	**Mean**	**s.d**	**p**
Sweeping coffee	0.06	773.73	3.12	21.12	75.14	**< 0.001**
Others	0.05	295.76	0.06	4.45	20.10	V > O

V → sweeping coffee *O → Others: unripe, natural cherry, raisin/float, husked cherry*
p < 0.001 – t student test

Table 2. Comparison of OTA contamination between different types of coffee.

	Type of coffee					
OTA	T_1	T_2	T_3	T_4	T_5	T_6
≤ 5ng/g	79.4	90.7	98.9	82.4	85.5	58.1
> 5 ng/g	20.6	9.3	1.1	17.6	14.5	41.9
Total	**100.0**	**100.0**	**100.0**	**100.0**	**100.0**	**100.0**

T_1 →Float *T_2 → Natural Cherry* *T_3 → Husked cherry* *T_4 →Unripe/Natural Cherry*
T_5 → Mixture *T_6 → Sweeping (Varrição) ($p < 0.001$ - χ^2 Qui square test)*

Regarding the type of harvest (mechanical and manual) it was observed a strong tendency ($p < 0.052$, χ^2 Qui square test) of having higher OTA contamination in the samples manually harvested. Upon the occurrence of higher content of OTA on coffees, which have been collected mechanically, it is not believed to be due to the harvesting itself, but to disturbances caused along the flow of drying, due to the dimensioning of drying terraces and dryers.

The manual or mechanical stripping directly on the soil has shown highly harmful to coffee increasing significantly ($p < 0.001$, χ^2 Qui square test) the levels of OTA contamination when compared to those stripped on a cloth, on a wide basket or on a sieve. That is a practise of harvesting that has been abandoned in Brazil, and replaced by a harvest on cloth or plastic, and even by a mechanical process. The practice of it is characterised by collection of all the fruits of the tree and the lifting of these fruits together with the coffee beans already fallen down on the soil longer before contributing in this way to OTA contamination.

No difference in OTA contamination was observed due to use of dryers in combination or not with terrace ($p < 0.541$, χ^2 Qui-square) as expected from any of these processes, since well conducted.

However, the type of terrace influences the levels of OTA contamination as can be observed in Table 3. Asphalt (mud) terraces contributed most to the production of OTA, followed by earth terrace and cement terrace. The asphalt (mud) terrace has been adopted by small coffee growers in substitution to earth, rather because of their low cost. So, this paradox of improvement of quality of terraces attributed to the asphalt (mud) and the present data finds explanation in the lack of conservation on the surface of the terrace, to deficient handling practices exercised by small coffee planters still not so well conscious of the necessary good practices. The asphalt terrace has some problems related to conservation of the surface, and along a few years of use, it turns to present frequently cracks and holes not allowing a proper cleaning of the surface thus facilitating the fungi growth (hot spots) and OTA contamination. Earth surface terraces are of difficult handling during rain periods, and this can be the explanation for the elevated number of contaminated samples. Regarding the cement terraces, which predominate in the coffee activity in Brazil, no other explanation for such level of contamination but improper handling, cracks on the surface (poor conservation), the occurrence of rain falls or even the origin of the coffee (from sweeping and floats) already contaminated in the plantation site. At suspended and brick terraces no OTA formation was observed at levels above 5 ng/g. These latest two types of terraces are rather rare in Brazil. The suspended terrace, have nowadays been adopted by growers who search for a better quality for their products and correspond preferentially to cherry coffees and/or husked cherry coffees, therefore, less subjected to the occurrence of OTA. Hence, the zeal at handling, associated with the characteristics of being suspended can explain this good performance. The brick terraces, which have been preferred, as compared to cement terraces, have shown a good result. This diversity of factors turns the explanation of the influence of type of drying terrace on the occurrence of OTA a rather complex issue.

Table 3. Influence of the Type of Terrace in the OTA contamination.

	Terrace					
OTA	**Earth**	**Cement**	**Asphalt (mud)**	**Suspended**	**Brick**	
≤ 5ng/g	82.7	86.8	62.0	100.0	100.0	
> 5 ng/g	17.3	13.2	**38.0**	0.0	0.0	
Total	100.,0	100.0	100.0	100.0	100.0	

p < 0.001 (p refer to Fisher test) *Suspended = Brick < (Cement) < Earth < Asphalt*

Another factors were evaluated concerning the influence in the OTA contamination. Among them altitude, irrigation, fungicides, harvest time, shading, wagging, topography, rainfall. It was notice a trend of smaller incidence of OTA as the altitude increased ($p < 0.01$, Fisher test). Coffee plantation in lower altitudes, with higher temperatures provide a more precocious ripening of the coffee, and under these conditions, at full sunlight, one can observe more incidence and sunray effects on the top of the tree increasing the percentage of dry/raisin coffee on the tree, that associated to a poor post-harvest management is likely to promote a higher content of OTA. Concerning the influence of the type of irrigation system in the OTA contamination, it was observed no significant difference in the OTA contamination of samples due to ordinary irrigation systems. However, on the "central pivot" system, a significant reduction of OTA occurrence ($p < 0.01$, Fisher t) was noticed which was not interpreted as the cause for the low levels of OTA. Plantations in the areas with such system "central pivot" are considered highly technical and conducted according to good agricultural practices (good harvest, post-harvest, drying and storage management). All this, associated to low relative air humidity conditions and absence of rainfalls, which are prevailing in the harvest period in these regions, provides the production of coffees practically free from OTA, being, most of them, husked cherries (36.2%). No influence of fungicides, harvest time, shading, wagging, topography, and rainfall) on the OTA contamination was determined.

Part 2

The highest occurrence of defects were: sour (98.3%), immature (85.0%), black (80.0%), insect-damaged (85.0%), broken (75.0%), shell and its pulp (88.3%), green (85.0%), black/immature (60.0%), insect-damaged - bluish (53.3%), malformed (41.7%), fox silver skin (25.0%), bean in parchment (22.3%), dried cherry-pod (15.0%), sticks/stalks (8.3%), husk (5.0%), pulp-nipped (3.3%), stones (1.7%). Concerning the variety of defects in the samples, it was observed that 66.7% of samples showed at least 9 types of defects.

The defect-free coffee sample (clean) contributed with around 80.0% of the composed sample (4 kg) overall mass and with 45.9% of the OTA contamination. Although the picked-up sample has contributed with 45.9% of OTA contamination the mean (2.97 ng/g) and median (1.77 ng/g) were relatively low. On the other hand, the sour defect contributes with 6.9% of the sample overall mass and with 25.3% of OTA contamination. The OTA contamination level for this defect showed a median of 4.8 ng/g and a mean of 79.8 ng/g. Following, the insect damaged - bluish defect, which contributes with 1.5% of the overall mass of the 4 kg sample and with 7.4% of OTA contamination with a median of 0.1 ng/g and a mean of 27.8 ng/g. The insect damaged and black defects contribute with 4.9% and 3.1% of OTA contamination, respectively.

From this result the overall sample contamination (composed sample) was re-calculated, by eliminating the main defects (sour, insect damaged - bluish defect, insect damaged and black), which was denominated as composed without defects.

Although the picked-up sample, sour, insect damaged - bluish, insect damaged, black, malformed, broken contribute the most to the presence of OTA contamination in coffee, the defects "black, stick/stalk, bean in parchment and insect damage-bluish" were more present in samples with contamination > 5 ng/g with 95% confidence ($p < 0.05$, Fisher test). The presence of these defect in coffee may be consider as indicative of OTA contamination in coffee.

Comparing the OTA contamination determined in the original sample (1 kg) and in the composed sample (4kg), a significant difference was not identified, although a reduction in the value of contamination was observed (Figure 2). On the other hand, significant differences (t student test, $p < 0.05$) were observed, when comparing the OTA contamination (mean) between the clean (picked-up) x defect, clean (picked-up) x original, clean (picked-up) x composed and clean (picked-up) x composed defect-free, showing that the presence of defects increase the levels of OTA contamination in coffee (Figure 2). From the comparison of OTA contamination in the composed sample and in the composed sample without the main defects it was observed that in fact the presence of the following defects: sour, insect damaged and insect damaged-bluish and black beans significantly increase OTA contamination (t student, $p < 0.05$).

It was possible to determine the degree of concordance between the size (1 kg or 4 kg) and type of sample (picked-up, original and composed) on the number of samples with contamination ≤ 5 ng/g and > 5 ng/g (Kappa test). Considering the cut off of 5 ng/g, it was observed that the percentage (78.3%) of the picked-up samples with contamination ≤ 5 ng/g when compared with the original samples (65%) showing that the absence of defects in fact contribute to reduce the contamination in coffee (Kappa = 0,44, $p < 0.001$).

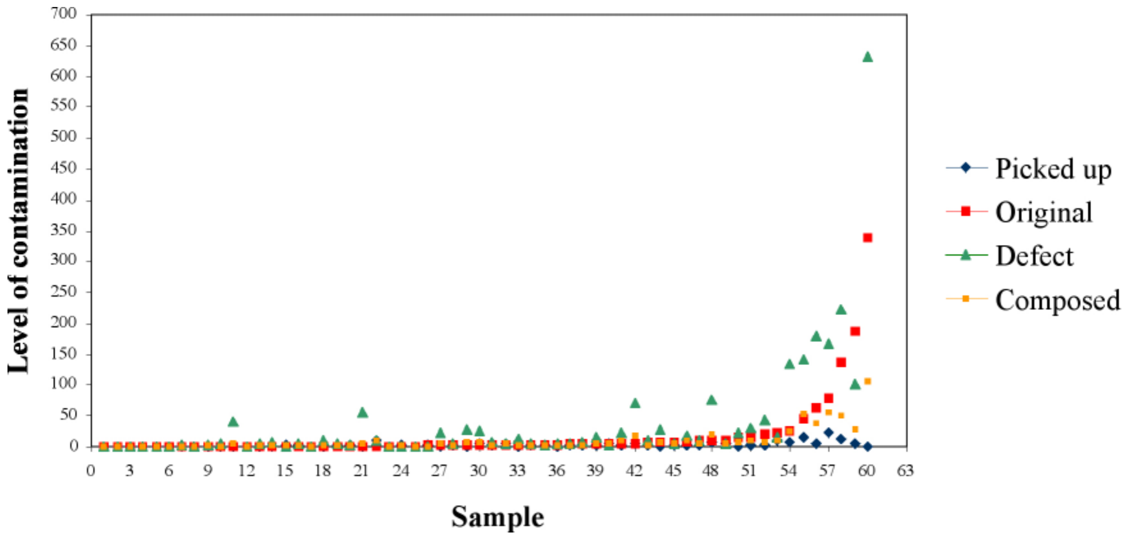

Figure 2. OTA contamination level in the original, clean, composed samples and in the defect.

Less impact was observed in the number of samples with OTA contamination ≤ 5 ng/g due to sample sizes of 1 kg (65%) and 4 kg (60%) (Kappa = 0.61, $p < 0.001$). The elimination of the main defects (sour, insect damaged and insect damaged-bluish and black beans) from the 4 kg samples increased to some extent the number of samples with OTA contamination ≤ 5 ng/g when compared to the 1 kg sample (Kappa = 0,53, $p < 0.001$).

There was no correlation of the quantity of defects and the percentage of mass with defect with the original and composed contamination (Pearson correlation).

CONCLUSION

From the univariate analysis it was possible to identify independent aspects (effects) of pre and post harvest that concur to the incidence and levels of OTA contamination in coffee. The multivarite analysis (logistic regression) indicates that besides the significant independent factors determined in the univariate analysis, the presence of OTA contamination with levels > 5 ng/g in coffee samples was (Central Pivot) influenced by the type of irrigation, the harvest method i.e. the contact of coffee with soil during harvest and the type of terrace. As discussed before the "Central Pivot" represents here the highly technical processing system conducted according to good agricultural practices showing the coffee processed under good management system are less susceptible to OTA contamination.

The presence of defects impacted significantly and negatively the incidence and levels of OTA in coffee. Among the defects the sour, insect damaged - bluish, insect damaged, black, malformed and broken were the ones that most contribute to the incidence and levels of OTA contamination in coffee. The defects "black, stick/stalk, bean in parchment and insect damage-bluish" were more present in samples with contamination > 5 ng/g.

No correlation between the quantity of defects and the percentage of mass with defect with the OTA contamination in both original and composed sample was determined.

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