INCIDENCE AND DISTRIBUTION OF FILAMENTOUS FUNGI DURING FERMENTATION, DRYING AND STORAGE OF COFFEE (COFFEA ARABICA L.) BEANS

Cristina Ferreira Silva¹; Luis Roberto Batista²; Rosane Freitas Schwan¹*

¹Departamento de Biologia, Universidade Federal de Lavras, Lavras, MG, Brasil; ²Departamento de Ciências dos Alimentos, Universidade Federal de Lavras, Lavras, MG, Brasil.

Submitted: August 12, 2007; Returned to authors for corrections: May 08, 2008; Approved: July 14, 2008.

ABSTRACT

The objective of this work was to isolate and characterize filamentous fungi present in different stages of harvest, fermentation, drying and storage of coffee beans processed by natural method. The cherries were hand-picked and then placed on a cement drying platform where they remained until reached 11% of humidity. Microbial counts were found in all samples during fermentation and drying of the coffee beans. Counts of fungi in the coffee cherries collected from the tree (time 0) were around $1.5 \times 10^3$ CFU/g. This number increased slowly during the fermentation and drying reaching values of $2 \times 10^5$ CFU/g within 22 days of processing. Two hundred and sixty three isolates of filamentous fungi were identified. The distribution of species during fermentation and drying was very varied while there was a predominance of *Aspergillus* species during storage period. The genera found were *Pestalotia* (4), *Paecelomyces* (4), *Cladosporium* (26), *Fusarium* (34), *Penicillium* (81) and *Aspergillus* (112) and comprised 38 different species.

Key-words: Fermentation, filamentous fungi, coffee, toxigenic fungi

INTRODUCTION

Coffee quality is evaluated by key factors including the selection of the *Coffea arabica* variety, climatic conditions during growth, processing method and storage conditions. The main aim of coffee processing is removal of the pulp, mucilage, parchment and silver skin surrounding the coffee beans, which leaves the so called ‘green’ coffee beans. In Brazil and Ethiopia and for robusta coffees generally, the dry or natural method of fermentation is usually used. During the natural processing, coffee fruits are spread on the ground (earth, concrete or tarmac) in layers approximately 10 cm thick, heaped at night and respread each morning. During the course of 10-25 days of sun drying, the natural microbial fermentation that occurs can influence the final quality of the product (32). Microbial contamination can occur in the cherries and during harvesting, fermentation, drying and storage coffee beans (32). Bacteria, yeasts and filamentous fungi have been already reported in the pulp and beans of coffee processed in Brazil, India, Hawaii, Congo, Argentina, Colombia, Costa Rica, Ethiopia and Mexico (2,12,30,32). Filamentous fungi predominate at the end of the processing and during storage, and may affect the quality and safety of the final product due to production of mycotoxins (4,6,34,35). Several studies have reported the occurrence of toxin-producing fungi and ochratoxin in green coffee beans (4,18,20,26, 28). A survey on stored green coffee beans from various origins has shown that coffee samples from African origin have significantly higher levels of OTA than those from America and Asia (21). The aim of this work was to detect the occurrence of filamentous fungi present in the different stages of coffee production by natural method including harvest, fermentation, drying and storage of beans.

MATERIALS AND METHODS

Sampling

One hundred and eight kg of beans from *Coffea arabica* var. Acaiá were collected from a farm located 750 to 800 m above
sea level in Lavras, State of Minas Gerais, Brazil. The hand
picked red cherries were fermented and dried on a concrete
platform for about 25 days until reaching humidity of 11-12%.
The beans were then packed in either polystyrene bags or jute
sacks and stored in a cold chamber at 3°C for 136 days, when
the relative humidity reached 90%.

Isolation and identification of filamentous fungi
Every 48 h, three samples of two hundred beans were
aseptically removed from the stored packages and placed in
sterile flasks and transferred to the laboratory in an ice box. The
beans were transferred to a bottle containing 1800 ml of saline
peptone diluent (0.1% of peptone, 0.5% of NaCl, 0.03%
Na₂H₂PO₄). After 15 min mixing, ten-fold dilutions were prepared.
Appropriate dilutions were spread on triplicate plates of DG18
(Dicloran Glycerol 18%) agar (11) containing 100 mg/L
chloramphenicol and 50 mg/L chlortetracycline to inhibit
bacterial growth. Plates were incubated at 28°C for 5 days, and
the colonies were counted and expressed as CFU/g.

For identification, the isolates were purified by streaking in
Malt Extract agar (Merck) according to Christensen (8) for species
of the Circumdati section, Christensen (9) for species of the
Flavi section, Klich and Pitt (14) for species of the Nigri and
Aspergillus section, Pitt (24) for species of the Penicillium genus
and Klich (13) for species of the Versicolores section. Further
support for fungi identification was found in Raper and Fennell
(27), Booth (5), Nelson et al. (19), Samson et al. (29) and Pitt and
Hocking (25).

RESULTS AND DISCUSSION

Coffee cherries and beans are subjected to contamination and
consequent colonization by microorganisms during different phases of development, harvesting, preparation,
transport and storage. Microbial counts were found in all
samples during fermentation and drying of the coffee beans.
Counts of fungi in the coffee cherries collected from the tree
time 0) were around 1.5 x 10³ CFU/g (Table 1). Most papers on
coffee contamination reported microbial counts from a mixture
of green (immature), red (mature), over mature, dark brown
and shriveled cherries (3,4,31,32,35). In this work, the fungi
population in hand-picked mature cherries increased slowly
during fermentation and drying, reaching values of 2 x 10⁵
CFU/g within 22 days of processing, when the beans contained
about 11% humidity. The highest count of filamentous fungi
was found at the 20th day of fermentation and drying. This
growth of fungi was probably due to reduction of water activity
which inhibits the growth of other microbial groups e.g.
bacteria and yeasts (Table 1). On the twentieth day of drying,
the humidity level and the water activity were 12.90% and
0.63, respectively. Two days later, the humidity in the grains
decreased and reached the ideal 11%, which corresponded to

Table 1. Total counts of filamentous fungi, relative humidity and 
water activity of the coffee grains during fermentation and 
drying.

| Time (days) | Counts (CFU/g) | Relative humidity (%) | Water activity |
|------------|----------------|-----------------------|----------------|
| 0          | 1.5 x 10³      | 67.45                 | >0.85          |
| 2          | 2.8 x 10³      | 60.83                 | >0.85          |
| 4          | 5.9 x 10³      | 38.85                 | >0.85          |
| 6          | 7.6 x 10³      | 29.35                 | >0.85          |
| 8          | 2.0 x 10⁴      | 28.56                 | >0.85          |
| 12         | 4.0 x 10⁴      | 19.72                 | 0.82           |
| 14         | 6.8 x 10⁴      | 19.30                 | 0.82           |
| 16         | 9.0 x 10⁴      | 19.70                 | 0.82           |
| 18         | 1.7 x 10⁵      | 15.78                 | 0.71           |
| 20         | 2.0 x 10⁵      | 12.90                 | 0.63           |
| 22         | 1.6 x 10⁶      | 11                    | 0.52           |

Table 2. Fungi population, relative humidity and water activity 
in green coffee grains stored in polystyrene and jute sacks.

| Time (days) | Type of packing | Counts (CFU/g) | Relative humidity (%) | Water activity |
|------------|-----------------|----------------|-----------------------|----------------|
| 40         | polystyrene     | 3.7 x 10³      | 13.05                 | 0.63           |
|            | jute            | 6.0 x 10⁴      | 17.90                 | 0.82           |
| 84         | polystyrene     | 1.2 x 10⁵      | 12.90                 | 0.63           |
|            | jute            | 9.7 x 10⁴      | 19.00                 | 0.82           |
| 136        | polystyrene     | 0.7 x 10⁵      | 12.50                 | 0.63           |
|            | jute            | 1.8 x 10⁶      | 19.0                   | 0.82           |
fungi species. These differences found in the number and diversity of fungi in the coffee beans could be related with the long period of fermentation and drying (15-25 days). This high frequency and diversity of fungi in green coffee samples analyzed at different stages of maturation and processing was also reported by Urbano et al. (37) and Pardo et al. (21).

A high diversity of fungi species was observed in cherries collected from trees. Fourteen different species belonging to genera Cladosporium, Fusarium, Pestalotia, Paecilomyces and Penicillium were detected (time 0 - Table 3). After the second day of fermentation, the total number of fungi increased slightly but there was a decrease in the number of identified species (Tables 1 and 3).

The microbial diversity on the surface of coffee cherries and beans in the South region of Minas Gerais State was also reported by Silva et al. (32). Aspergillus, Penicillium, Fusarium and Cladosporium are known as natural coffee contaminants, and are present from the field to the warehouse (18,22,32). The humidity and chemical composition of the coffee beans, environmental conditions and crop and product management can influence development of microorganisms and their metabolic activity.

Table 3. Species of filamentous fungi detected in cherries and green coffee during different phases of processing: fermentation, drying and storage in polystyrene and jute sacks.

| Time (days) | Species of filamentous fungi |
|-------------|-----------------------------|
| **Fermentation and drying** | |
| 0 | Cladosporium cladosporioides (seven), Fusarium lateritium (four), F. solani (four), F. illudens (two), F. moniliforme (sin. verticilloides) (two), F. nivale (one), Pestalotia sp. (three), Paecilomyces sp. (one), Penicillium minioluteum (three), P. roqueforti (one), P. solitum (one), P. funiculansum (two), P. brevicaespactum (two), P. chrysogenum (one) |
| 2 | C. cladosporioides (three), Paecilomyces sp. (one), P. minioluteum(one), P. crustosum (one) |
| 4 | C. cladosporioides (two), F. solani (two) |
| 6 | C. cladosporioides (one), F. solani (two), P. purpureogenum (two) |
| 8 | C. cladosporioides (five), Aspergillus flavus (one), F. illudens (one), Pestalotia sp. (one) |
| 12 | C. cladosporioides (one), A. flavus (one), Paecilomyces (two), P. fellutanum (one), P. corylophilum (two), P. solitum (one) |
| 14 | A. flavus (one), P. roqueforti (one), P. expansum (one), P. citrinum (one), P. janthinelum (one), P. fellutanum (one), P. brevicaespactum (nine), P. chrysogenum (two) |
| 16 | F. solani (three), F. illudens (two), F. xylarioides (two), F. stilboides (one), F. concolor (one), F. equiseti (one), P. solitum (one) |
| 18 | C. cladosporioides (two), A. flavus (one), P. roqueforti (six), P. citrinum (one), P. brevicaespactum (one), P. crustosum (one) |
| 20 | A. ochraceus (twelve), A. flavus (three), F. xylarioides (one), F. trincictum (one), P. brevicaespactum (twelve), P. roqueforti (two), P. aurantiogramiseum (one), P. waksmanii (one), P. citrinum (one), P. minioluteum (one), P. solitum (one) |
| 22 | A. flavus (five), A. niger (twenty), A. tamari (one), A. sydowii (one), F. lateritium (one), P. aurantiogramiseum (one) |
| **Storage** | |
| 40 days (polystyrene bags) | A. flavus (six), P. citrinum (two), P. corylophilum (one), P. chrysogenum (one), P. roqueforti (one) |
| 40 days (jute sacks) | A. flavus (one) |
| 84 days (polystyrene bags) | A. flavus (two), P. brevicaespactum (one), P. viridicatum (one), P. citrinum (one) |
| 84 days (jute sacks) | C. cladosporioides (five), F. concolor (one), P. roqueforti (one), P. citrinum (one), P. solitum (one) |
| 136 days (polystyrene bags) | A. flavus (eleven), A. niger (nine), F. lateritium (one), P. citrinum (two) |
| 136 days (jute sacks) | C. cladosporioides (two), A. flavus (twenty one), A. niger (thirteen), A. foetidius (one), A. dimorphicus (two), F. lateritium (one), P. citrinum (one), P. implicatum (one), P. crustosum (one), P. waksmanii (one) |
Cladosporium cladosporioides was the most frequent specie found on cherries in the tree (time 0), on the grains during the fermentation until 12th day and in grains stored in jute sacks for 84 and 136 days (Table 3). Magan and Lacey (15) studied the colonization in coffee grains with Cladosporium cladosporioides, Fusarium culmorum, species of Aspergillus and Penicillium brevicompactum and P. roqueforti, and observed that the two first species were present in the field and the two last species were colonizers of dried coffee grains during storage. The authors observed that the growth of Cladosporium cladosporioides was inhibited by species of Fusarium, Penicillium and Aspergillus. Comparing the species of fungi present in the coffee beans, the highest incidence of Cladosporium cladosporioides was detected when competitors were absent or present in a reduced number (Table 3). These results are in agreement with those reported by Pereira et al. (22), who observed that this Cladosporium cladosporioides specie corresponded to 100% of the identified isolates in the cherries and grains of coffee. There is evidence that this fungus is common in coffees of good quality (22).

The Fusarium species was detected in cherries on the tree, in the beans during the process of fermentation and drying and in the beans stored both in jute sacks (84 and 136 days) and in polystyrene packs (136 days) (Table 3). Fusarium lateritium and F. solani were more frequent than other Fusarium species. F. lateritium also was isolated in cherries on the 22nd day of drying and at the end of storage in both types of packages (Table 3). F. illudens was observed in cherries in the tree (time 0), in grains on the 8th and 16th days of fermentation and drying. Although the majority of the toxigenic fungi reported in coffee are Aspergillus and Penicillium (4,17,31,35,38), Fusarium species, involved in the coffee processing, were also reported by Silva et al. (32) and Pimenta and Chalfoun (23), possibly as mycotoxin producers.

Isolates belonging to the genus Penicillium were observed in all samples, during fermentation, drying and also during the storage in the two types of packages (Table 3). This genus presented the highest diversity of species as 17 different species were identified (Table 3). P. brevicaespactum was identified in cherries in the tree, in grains on the 14th, 18th and 20th days of drying and in grains after 84 days of storage in polystyrene packs. Among species of Penicillium studied by Magan and Lacey (15), P. brevicaespactum was the most prevalent due to release of metabolites in the substratum that had inhibited or limited the growth of other species of fungi. In the present study, the second specie most frequent belonging to the genus Penicillium was P. roqueforti, representing 15.2% of the isolates. This specie was identified in coffee beans on the 14th, 18th and 20th days of fermentation and drying of the beans after 40 days of storage in polystyrene bags and after 84 days of storage in jute sacks. Magan and Lacey (15) reported that this specie inhibits the growth of other Penicillium species. P. citrinum was not identified in cherries in the tree but identified in the grains containing humidity between 19.49% and 13.74% (14, 18 and 20 days of fermentation). During the storage, this specie was detected in jute sacks (84th day) and in polystyrene packing. Species of Penicillium were identified in coffee beans during processing, especially in the last two days of drying (a, of 0.71 and 0.63, respectively), being therefore xerophilic fungi. P. citrinum was found in coffee cherry in agreement with its requirement for high water activity (aw) for growth. Our results indicated that P. citrinum occurred only when the water activity was about 0.84. Besides being xerophilic, P. roqueforti is a psychrophilic fungus (25), so its presence can be explained by the low temperature of storage chamber. Some species of Penicillium found in the coffee grains, such as P. roqueforti, P. citrinum, P. chrysogenum, P. crustosum, P. aurantiogriseum, P. funiculosus, P. janthinellum, P. expansum can produce mycotoxins, and their presence in the grains is important because they compromise the quality and safety of the product. However the presence of toxigenic fungi does not indicate that mycotoxins were also present, as evidenced by Batista et al. (3) and Silva et al. (31).

The presence of P. brevicaespactum, P. citrinum, P. aurantiogriseum and P. expansum found in this study agree with results reported by Batista et al. (4) and Mislivec et al. (17), who cited these species as the principal members of Penicillium genus found in coffee beans. The high frequency of Aspergillus (96%) and Penicillium (50%) species confirms the widespread natural contamination of coffee with these fungi (1,4,31,35).

One hundred and twelve isolates of Aspergillus had been identified: A. flavus (53 isolates), A. niger (42 isolates), A. ochraceus (12 isolates), A. tamarii (1 isolate), A. sydowi (1 isolate), A. foetidius (1 isolate), A. dimorphicus (2 isolates). These species were detected at the 8th day of drying of the beans and during storage, representing 59.6% of the total isolates. A. flavus, the most frequent specie, was presented in the 8th day of drying and also in the 12th, 14th, 18th, 20th and 22nd days of fermentation, and at the end of storage in the two kinds of packing. Aspergillus niger represented 37.8% of the isolates of the genus Aspergillus, being only found in the last day of fermentation/drying of the beans (22nd day), and in beans stored in jute and polystyrene sacks (Table 3). A. ochraceus was found only at the 20th day of fermentation, not being detected during storage. Aspergillus species have been frequently reported in beans and stored grains of coffee (3,4,6,7,17,32,33,35,37). Besides compromising the quality of the product, the presence of Aspergillus may affect their safety due to production of secondary metabolites toxic to man and animals (mycotoxins). Aspergillus ochraceus, A. niger and A. carbonarius had been also found in samples of grains of coffee by Taniwaki and collaborators (35). Although A. carbonarius is a common contaminant of wines (10), its presence in stored coffee beans is rare in Brazil (35).

Biotic and abiotic parameters such as water activity (aw) and interactions among fungi determine the extent of
colonization and the microbiota found in coffee beans (16). The ability of fungi to germinate, grow and sporulate in coffee grains is also dependent on $a_w$, temperature and intergranular composition of gases (36). The biotic and abiotic factors that influence the establishment of groups of microorganisms (bacteria, yeasts and filamentous fungi) in coffee during natural processing are not known. However, it is known that maize with high humidity can become contaminated by microorganisms that interact during post-harvesting and storage. Species of filamentous fungi can interact in different ways, including the production of metabolites that can influence the settlement of specific strains (15). The interaction among filamentous fungi observed in maize could be extended for those observed in coffee beans containing 67.45% humidity. According to Pitt and Hocking (25), *Aspergillus* competes for substrate with *Fusarium* and *Penicillium*, and its incidence increases only in environments with high temperature and low water activity, which are the ideal conditions found in the final stages of processing and drying during storage of coffee. Many species of fungi identified in this work had already been detected in cherries and grains of coffee (3,17,31,32).

In this work, the number of isolated fungi in coffee grains increased during storage, where *Aspergillus flavus* and *A. niger* predominated. During storage, the number of species isolated in samples from jute sacks was higher than in samples stored in polystyrene bags. The polystyrene bags are less permeable in samples from jute sacks was higher than in samples stored in polyesterene bags. The polystyrene bags are less permeable and re-absorption of water occurs in lesser extent that in the jute sacks. Despite the high humidity of the green coffee grains, humidity and temperature were not favorable for the growth of potentially toxigenic species such as *Aspergillus flavus* and *A. ochraceus*. The minimum water activity for production of aflatoxin by *A. flavus* is 0.82, which corresponds to approximately 18.4% of humidity. For *A. ochraceus*, the minimum water activity to produce ochratoxin is 0.85 corresponding to approximately 20% of humidity in coffee grains (34). The minimum and maximum temperature of growth for *A. flavus* range between 6 and 10°C and between 25 and 37°C, respectively, however for aflatoxin B$_1$ and B$_2$, production the ideal temperature is between 16 and 31°C. For *A. ochraceus* the minimum and maximum temperature of growth is between 8 and 12°C and between 24 and 31°C, respectively, while for ochratoxin production, the temperature should be between 25 and 31°C (34). It is interesting to observe that despite the isolation of these fungi in stored coffee grains, ideal temperature for development of the species and for production of toxins is much higher than the temperature in the cold storage chamber (3°C). Although several species of toxin producing fungi were found during coffee processing neither ochratoxin nor aflatoxin were detected in any sample (data not shown).

The species identified in this study are among the most common species of fungi present in storage environments. They can tolerate growth in different substrates and environmental conditions, and their complete elimination is difficult. However, the use of good hygiene practices and good management of the beans during processing can minimize the production of the mycotoxins in coffee.

**ACKNOWLEDGEMENTS**

Conselho Nacional de Desenvolvimento Científico e Tecnológico do Brasil (CNPq) and Fundação de Amparo a Pesquisa do Estado de Minas Gerais (FAPEMIG) are acknowledged for financial support. CFS thanks CAPES (Coordenação de Aperfeiçoamento de Pessoal de Nível Superior) for the scholarship during her Ph.D course.

**RESUMO**

**Incidência e distribuição de fungos filamentosos durante a fermentação, secagem e armazenamento de frutos e grãos de café (Coffea arabica L.)**

O objetivo deste estudo foi isolar e caracterizar fungos filamentosos presentes em diferentes estágios de beneficiamento de café processado pelo método natural, incluindo: colheita, fermentação, secagem e armazenamento. O café cereja foi colhido manualmente e então colocado em uma plataforma de cimento, onde permaneceu até atingir 11% de umidade. A contagem microbiana foi realizada em todas as amostras durante a fermentação e secagem do café. A população de fungos filamentosos no café cereja ainda nos pés (tempo 0) foi em torno de 1,5 x 10$^3$ UFC/g. Este número aumentou vagarosamente durante a fermentação e secagem, alcançando valores de 2 x 10$^4$ UFC/g em 22 dias do processamento. Duzentos e sessenta e três isolados de fungos filamentosos foram identificados. A distribuição das espécies durante fermentação e secagem foi bastante variada, mas no armazenamento dos grãos ocorreu o predomínio de espécies de *Aspergillus*. Foram encontradas 38 espécies de fungos distribuídas nos seguintes gêneros: *Pestalotia* (4), *Paecelomyces* (4), *Cladosporium* (26), *Fusarium* (34), *Penicillium* (81) e *Aspergillus* (112).

Palavras-chave: Fermentação, fungos filamentosos, café, fungos toxigênicos

**REFERENCES**

1. Abdel-Hafez, A.I.I.; EL-Maghraby, O.M.O. (1992). Fungal flora and aflatoxin associated with cocoa, roasted coffee and tea powders in Egypt. Cryptogam. Mycol., 13, 31-45.
2. Avallone, S.; Guyot, B.; Brillouet, J.M.; Olguin, E.; Guiraud, J.P. (2001). Microbiological and biochemistry study of coffee fermentation. Curr. Microbiol., 42, 252-256.
3. Batista, L.R.; Chalfoun, S.M.; Prado, G. (2001). Identificação de espécies toxigênicas de *Aspergillus* associadas aos grãos de café armazenados. Rev. Bras. Armaz., 3, 11-16.
4. Batista, L.R.; Chalfoun, S.M.; Prado, G.; Schwan, R.F.; Wheals, A.E. (2003). Toxigenic fungi associated with processed (green) coffee beans (Coffea arabica L.). Int. J. Food Microbiol., 85, 293-300.

5. Booth, C. (1971). The genus Fusarium: Survey of wealth in mycological Institute, p. 237.

6. Bucheli, P.; Meyer, I.; Pittet, A.; Vuatag, G.; Viani, R. (1998). Industrial storage of green robusta under tropical conditions and its impact on raw material quality and Ochratoxin A content. J. Agric. Food Chem., 46, 4507-4511.

7. Bucheli, P.; Taniwaki, M.H. (2002). Research on the origin, and on the impact of post-harvest handling and manufacturing on the presence of ochratoxin A in coffee. Review Food Addit. Contam., 19, 655-665.

8. Christensen, M. (1982), The Aspergillus ochraceus Group: Two new species from western soils and a synopte key. Mycologia, 74, 210-225.

9. Christensen, M. (1981). A Synoptic key and evaluation of Specie in the Aspergillus flavus group. Mycologia, 73, 1056-1084.

10. Esteban, A.; Abarca, M.L.; Bragulat, M.R.; Cabañes, F.J. (2006). Dichloran glycerol medium for enumeration of xerophilic fungi from low moisture foods. App Environ. Microbiol., 23, 634-640.

11. Hocking, A.D.; Pitt, J.L. (1980). Dichloran glycerol medium for enumeration of xerophilic fungi from low moisture foods. App Environ. Microbiol., 23, 656-660.

12. Jones, K.L.; Jones, S.E. (1984). Fermentations involved in the production of cocoa, coffee and tea. Prog. Ind. Microbiol., 19, 411-56.

13. Klich, M.A. (1993). Morphological studies of Aspergillus Section Versiculares and related species. Mycologia, 85, 100-107.

14. Klich, M.A.; Pitt, J.I. (1988). A Laboratory guide to common Aspergillus species and their Teleomorphs. CSIRO Division of Food Science and Technology, North Ryde, Sydney, New South Wales.

15. Magan, N.; Lacey, J. (1984). Effect of water activity, temperature and substrate on interactions between field and storage fungi. Trans. Br. Mycol. Soc., 82, 83-93.

16. Magan, N.; Lacey, J. (1988). Ecological determinants of mould growth in stored grain. Int. J. Food Microbiol., 7, 245-255.

17. Misilveci, P.B.; Bruce, V.R.; Gibson, R. (1983). Incidence of Toxigenic and other molds in green coffee beans. J. Food Prot., 46, 969-973.

18. Nakajima, M.; Tsubouchi, H.; Miyake, M.; Ueno, Y. (1997). Detection of aflatoxin B1 and ochratoxin A in coffee from different origins. J. Agric. Food Chem., 45, 969-973.

19. Nelson, P.E.; Toussoun, T.A.; Marsaras, W.F.O. (1983). Fusarium species - An Illustrated Manual for Identification, EUA, 193 p.

20. Otteneder, H.; Majerus, P. (2001). Ochratoxin A (OTA) in coffee: nation-wide evaluation of data collected by German Food Control 1995-1999. Food Addit. Contam., 18, 431-435.

21. Pardo, E.; Marín, S.; Ramos, A.J.; Sanchis, V. (2004). Occurrence of ochratoxinogenic Fungi and Ochratoxin A in green coffee from different origins. Food Sci. Technol. Int., 10, 45-50.

22. Pereira, R.T.G.; Pfenning, L.H.; Castro, H.A. (2005). Caracterização e dinâmica de colonização de Cladosporium cladosporioides (Fresen.) de Vries in frutos do cafeí (Coffea arabica L.) Ciência Agrotec., 29, 1112-1116.

23. Pinamena, C.J.; Chalfoun, S.M. (2001). Composição microbiana associada ao café com cacao e beneficiado colhido em diferentes estádios de maturação. Ciência Agrotec., 25, 677-682.

24. Pitt, J.I. (2000). A laboratory guide to common Penicillium species. 3 ed. CSIRO, 197 p.

25. Pitt, J.I.; Hocking, A.D. (1997). Fungi and Food Spoilage. 2.ed. Cambridge: Chapman and Hall.

26. Pittet, A.; Royer, D. (2002). Rapid, low cost thin-layer chromatographic screening method for the detection of ochratoxin A in green coffee at a control level of 10 μg/Kg. J. Agric. Food Chem., 50, 243-247.

27. Raper, K.B.; Fennell, D.I. (1965). The Genus Aspergillus. Baltimore: Williams and Wilkins.

28. Romani, S.; Sacchetti, G; Chaves Lopez, C.C.; Pinnavia, G.G; Rosa, M.D. (2000). Screening on the occurrence of ochratoxin A in green coffee beans of different origins and types. J. Agric. Food Chem., 48, 3616-3619.

29. Samson, R.A.; Reenen-Hoekstra, E.S.V. (1988). Introduction to foodborne fungi. Baarn: Centraalbureau voor Schimmelcultures, Institute of the Royal Netherlands Academy of Arts and Sciences.

30. Schwan, R.F.; Wheals, A.E. (2003). Mixed microbial fermentations of chocolate and coffee. In: Vincent Robert. (Org.) Yeasts in Food. Hamburg, Alemanha: Behr’s Verlag, vol. 1, pp. 426-459.

31. Silva, C.F.; Batista, L.R.; Schwan, R.F. (2003). Incidência de Aspergillus produtores de microtoxinas em frutos e grãos de café (Coffea arabica L.). Rev. Bras. Armaz., 7, 30-37.

32. Silva, C.F.; Schwan, R.F.; Dias, E.S.; Wheals, A.E. (2000). Microbial diversity during maturation and natural processing of coffee cherries of (Coffea arabica L.) in Brazil. Int. J. Food Microbiol., 60, 251-260.

33. Silva, C.F.; Schwan, R.F.; Dias, E.S.; Wheals, A.E. (2004). Microbiota presente em frutos e grãos de café (Coffea arabica L) despolido e natural - uma revisão. Ciência Tecnol. Alimen., 37, 22-28.

34. Taniwaki, M.H. (2006). An update on ochratoxigenic fungi and ochratoxin A in coffee. In Hocking AD, Samson RA, Pitt JI, Thранe, U (eds.). Adv. Food Mycol., 571, 189-202.

35. Taniwaki, M.H.; Pitt, J.I.; Teixeira, A.A.; Imanakka, B.T. (2003). The source of ochratoxin A in Brazilian coffee and its formation in relation to processing methods. Int. J. Food Microbiol., 82, 173-179.

36. Torres, M.R.; Ramos, A.J.; Soler, J.; Sanchis, V.; Marín, S. (2003). SEM study of water activity and temperature effects on the initial growth of Aspergillus Ochraceus. Alternaria Alternata and Fusarium Verticilloides on maize grain. Int. J. Food Microbiol., 81, 185-193.

37. Urbano, G.R.; Taniwaki, M.H.; Leitão, M.F.; Vicentini, M.G. (2001). Ochratoxin A in green coffee at a control level of 10 μg/Kg. J. Agric. Food Chem., 49, 677-682.

38. Vega, F.E.; Posada, F.; Peterson, S.W.; Gianfagna, T.J.; Chaves, F. (2006). Penicillium species endophytic in coffee plants and ochratoxin A production. Mycologia, 98, 31-42.