Food Research International 62 (2014) 353–358

Contents lists available at ScienceDirect

Food Research International

journal homepage: www.elsevier.com/locate/foodres

The mycobiota of coffee beans and its influence on the coffee beverage

B.T. Iamanaka [a,*], A.A. Teixeira [b], A.R.R. Teixeira [b], M.V. Copetti [c], N. Bragagnolo [d], M.H. Taniwaki [a]

[a] Food Technology Institute ITAL, Campinas, SP, Brazil
[b] Assicafé Assessoria e Consultoria Agrícola Ltda, São Paulo, SP, Brazil
[c] Federal University of Santa Maria — UFSM, Food Science Technology Department, Rural Science Center, Santa Maria, RS, Brazil
[d] University of Campinas — UNICAMP, School of Food Engineering, Department of Food Science, Campinas, SP, Brazil

ARTICLE INFO

Article history:
Received 30 November 2013
Accepted 23 February 2014
Available online 12 March 2014

Keywords:
Mycobiota
Penicillium sp.
Aspergillus section *Nigri*
Aspergillus westerdijikiae
Coffee
Sensorial analyses
Coffee beverage

ABSTRACT

The quality of coffee beverage is influenced by several factors, including the species or botanical variety of the beans, agricultural practices, harvesting, drying and storage techniques and also the preparation of the beverage. Apart from these, there is the input of microbial contamination during the processing of the beans. Numerous studies have demonstrated that fungi are important contaminants of coffee beans, especially just after harvesting and drying. However, the relationship between fungal contamination and the sensorial characteristics of the beverage has yet to be described. The aim of this research was to analyze the mycobiota of coffee beans collected from different stages of the coffee production chain and to correlate these data with the sensorial characteristics of the final beverage. Fungal infection of 22 coffee bean samples from the southwest of São Paulo state was analyzed. Samples were collected from the tree (mature cherries), from the ground, from the patio (mature, immature and dried floaters or overripe cherries from the tree) and from storage facilities. In general, coffee samples from this region showed high fungal infection and contamination was higher than 70% in about 45% of the samples. A high diversity of fungi was isolated from all the coffee samples analyzed and the most common were *Penicillium brevicompactum*, *Aspergillus* section *Nigri*, *Penicillium* sp. nov. (closely related to *Penicillium crustosum*) and *Fusarium* sp. Both *P. brevicompactum* and *Penicillium* sp. nov. were found at all processing stages, including in the cherries, showing that these fungi are naturally found in the coffee beans from this region. Floater coffee and coffee from the ground showed negative sensorial evaluation with attributes such as moldy, dirty and fermented and presented a high contamination by *Aspergillus* section *Nigri* and *Aspergillus westerdijikiae*.

1. Introduction

Coffee is subjected to several processes before it is consumed as beverage, and several factors contribute to its final quality, including the microbial population present. Considering that coffee beans provide a great variety of substrates that the coffee bean provides (husk, pulp and seed), the development of a diverse and complex microbiota is possible, including filamentous fungi, especially *Aspergillus*, *Fusarium* and *Penicillium* and also yeasts, lactic bacteria and pectolytic bacteria (Carvalho, Chagas, & Souza, 1997; Silva, Batista, Abreu, Dias, & Schwan, 2008).

Some studies have confirmed that filamentous fungi are the most common contaminants in coffee cherries after harvesting and during drying, which can increase the amount of defective beans (Carvalho et al., 1997; Illy & Viani, 2005; Taniwaki, Pitt, Teixeira, & Iamanaka, 2003; Urbano, Taniwaki, Leitão, & Vicentini, 2001). Taniwaki, Iamanaka, Copetti, Teixeira, and Teixeira (2006) compared the mycobiota of defective and healthy Arabica and Robusta coffee and notice a positive correlation between the presence of defects and the level of fungal infection. Samples with defective beans showed an increase of fungal infection from 18 to 33% in Arabica and from 92 to 98 % in Robusta coffee. The authors also studied the occurrence of ochratoxin A producing fungi in these samples and found that black and sour defective beans had the highest infection by *Aspergillus* section *Nigri* and *Aspergillus westerdijikiae* (Taniwaki, Teixeira, Teixeira, Copetti, & Iamanaka, 2014). The cause of these two defects is related to the extensive contact of these coffee beans with the soil, which can explain the high fungi infection. Ochratoxin A is a nephrotoxic and mutagenic toxin produced mainly by *A spergillus ochraceus*, *Aspergillus westerdijikiae*, *Aspergillus niger* and *Aspergillus carbonarius* in coffee (Joosten, Goetz, Pittet, Schellenberg, & Bucheli, 2001; Noonim, Mahakarnchanakul, Nielsen, Frisvad, & Samson, 2008; Taniwaki et al., 2003).

The contaminated beans can decrease the quality of the coffee beverage and change its sensorial characteristics, conferring undesirable attributes to the beverage. Currently there is no international standard available for sensorial evaluation of coffee beverage thus each country applies its own methodology, disregarding the differences between the conditions of beverage preparation of the exporting and consuming countries. In fact, some techniques have been developed

* Corresponding author at: Avenida Brasil, 2880, Campinas, SP Zip code 13070-178, Brazil.
E-mail address: beatriz@ital.sp.gov.br (B.T. Iamanaka).

http://dx.doi.org/10.1016/j.foodres.2014.02.033
0963-9969/

and recommended by the International Coffee Organization (ICO), aiming at establishing a descriptive sensorial standard terms that are easier for the consumer to understand, such as the Quantitative Descriptive Analyses (QDA), using trained teams (Meilgaard, Civille, & Carr, 1987; Stone & Sidel, 1985). Attributes such as those related to aroma (rubber, caramel, chocolate, floral, burnt, tobacco and others), taste (bitter, sour, sweet and salty), astringency and body are evaluated by the trained team. In the Brazilian internal market, coffee is classified by the Beverage Cup Test (Teixeira, 1970) and the main categories are: "mole" (pleasant and sweet taste) and "dura" (astringent but with no negative taste), which refers to a good quality beverage, and "riado" (chemical taste) which refers to a low quality beverage. QDA and other techniques can also be used to evaluate coffee sold in Brazil. However, the Espresso Test is used when coffee will be traded in the export market, specially to Italy. The espresso beverage is obtained by percolation of hot water under pressure through a cake of roasted ground coffee (Illy & Viani, 2005).

There is a lot of data establishing the relationship among different environmental conditions, coffee processing stages and sensorial quality (Barbosa et al., 2012; Bhumiratana, Adhikari, & Chambers, 2011; Borém et al., 2013; Kreuml, Majchrzak, Ploederl, & Koenig, 2013); but few have related the characteristics of the beverage with fungal contamination (Alves & Castro, 1993; Carvalho, Chalfoun, & Chagas, 1989). Considering the facts mentioned above, the objectives of this study were: (i) to evaluate the mycobiota of coffee beans at different stages of the production chain and, (ii) to investigate their impact on the quality of the beverage through sensory evaluation.

2. Material and methods

2.1. Coffee samples

Coffee samples from two different farms located in the southwest of São Paulo state (altitude 23°11′37″, longitude 49°23′02″, 646 m high, mean annual temperature of 20,1 °C and, mean annual rainfall of 1263 mm, IBGE, 2013) were evaluated. Approximately 2 kg (a total of 22 coffee samples) was collected from different stages of the coffee production chain: mature cherries from the tree (3), immature cherries (4), cherries from the ground (2), dried floaters (overripe cherries from the trees) (5) and coffee beans from storage facilities (8).

2.2. Water activity

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

2.3. Mycological analysis

Coffee beans were surface disinfected with 0.4% chlorine solution for 1 min, and then a total of 50 beans were plated directly onto Dichloran 18% Glycerol agar (Pitt & Hocking, 2009). The plates were incubated at 25 °C for 5 days and were visually analyzed for colony growth. After incubation all fungal colonies were inoculated on Czapek Yeast Autolyzate agar (CYA) (Pitt & Hocking, 2009) and purified for subsequent identification. The infection by each species or genera was expressed as the percentage of infection.

2.4. Identification of fungi

Isolates were grown on standard identification media, Czapek Yeast Autolyzate agar and Malt Extract agar and identified according to Klich and Pitt (1988), Pitt and Hocking (2009), Frisvad, Frank, Houbraken, Kuijpers, and Samson (2004) and Samson, Hoekstra, Thrane, Frisvad, and Andersen (2010). *Fusarium* sp. were grown monosporically in Carnation Leaf agar (CLA) plates and Potato Dextrose agar (PDA) slants for 10–14 days and were identified following Nelson, Toussoun, and Marasas (1983).

2.5. Sensory analysis

The beverage was evaluated by a trained team (Assicafé taster's group, São Paulo) made up of 5 trained judges. To evaluate the characteristics of the beverage, the Espresso test was carried out and three tasting tests were used according to the methodology described in Illy and Viani (2005): infusion, diluted espresso and espresso. The raw coffee was roasted at 220 °C for 5–6 min using a Probat gas roaster BRZ4. For the infusion test, 10 g of powder was added to 100 ml of water at 90 °C; and for the Espresso test, the beverage was prepared with 13 g of powder and 50 ml of water at 90 °C under pressure (9 bar) with a leaching time of 30 s. Diluted espresso was obtained by diluting (1:2) espresso with water at a temperature of 80 °C. All the three coffee preparations were submitted to sensory evaluation. The machine used to prepare the espresso was La Marzocco (EE-2G model, Italian brand).

The attributes evaluated by the experts in the tasting of espresso were: body, aroma, acidity, bitterness, sweetness and astringency. The final assessment of the beverage was described as positive or negative, after the team consensus decision. Moreover, the presence of positive flavors and aromas such as caramel, chocolate, almond, fruity and floral, and negative attributes including immature, fermented, woody, rancid, moldy, "riado", "rio" and smoky were evaluated.

3. Results and discussions

Table 1 shows the total fungi and the main species isolated from the southwest coffee samples, the results of the sensory analysis and water activity.

A high incidence and diversity of fungi were found and about 45% of the samples had fungal infection higher than 70%. The main species isolated were *Penicillium brevicompactum*, *Penicillium* sp. nov. (closely related to *Penicillium crustosum*), *Aspergillus* section *Nigri*, *A. westerdijikiae* and *Fusarium lateritium*.

Fifty percent of the analyzed samples showed negative sensorial evaluation, including all dried floaters (5 samples), coffee from the ground (2 samples), immature (3 samples) and one coffee sample from storage. All the dried floater coffee evaluated showed negative attributes such as fermented, "stinker" (strongly fermented), moldy and immature and all of them also showed fungal infection above 30%. The dried floater coffee is defined as the beans that become overripe on the tree. After harvesting dried floaters are separated by density in the tank, from the mature and immature cherries and are usually taken to the patio for natural drying. Among the fungal species isolated in these samples, a high incidence of *Aspergillus* section *Nigri* was found, varying from 0 to 34% of infection with an average of 15.2%.

The beverage prepared with beans from the ground (those which had contact with soil and dust) showed similar results to the ones found for the floater coffee beverage. They had a negative final evaluation and the characteristics moldy, "riado", (chemical taste), fermented, and highly bitter and in these samples, apart from the presence of *Penicillium* sp., a high infection by *Aspergillus* section *Nigri* (16 to 64%) and *A. westerdijikiae* (up to 30%) was verified. Fig. 1 shows the infection by *Aspergillus* section *Nigri* in coffee from the ground from the southwest of São Paulo.

P. brevicompactum was present in 96% of the samples including the cherries. The average infection by this species was 23.3%, ranging from 0 to 70%. The coffee with this species with fungal infection above 50% showed positive results for sensory evaluation results. The main attributes for this beverage were caramel aroma, floral, soft and sweet. Fig. 2 shows the coffee beans (from storage) infected with *Penicillium* sp.

Low occurrence of *Penicillium* sp. nov. (closely related to *P. crustosum*) was found. This species was present in 55% of the samples and had an average infection of 6.6%, ranging from 0 to 34%. Other genera of fungi