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Coffee Fermentation

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42.1 Introduction

Originally, coffee was grown in the Kaffa region in Ethiopia; later, in the early 17th century, the Dutch spread it throughout Europe. Coffee reached North America in the beginning of the 17th century and South and Central America in 1825. The habit of drinking coffee was developed in Arab culture, and the coffee beverage was known only for its properties as a stimulant. Coffee fruit was consumed raw and fed to herds as a stimulant while they were traveling. In 1000 AD, the Arabs began to prepare an infusion of coffee cherries, boiling them in water. The roasting process was developed only in the 14th century, and coffee finally became similar to today's beverage (ABIC 2010).

As a beverage, coffee is characterized as energizing and nonalcoholic, with potential beneficial effects on human health; it mainly acts as an antioxidant (Andueza et al. 2004). There are different types of coffee beverages characterized by different nuances in terms of body, aroma, acidity, and astringency.

One hundred species are associated with the genus *Coffea*, but only two species are agroeconomically important, *Coffea arabica* and *Coffea canephora*. *Coffea dewevrei*, *C. congensis*, *C. eugenioides C. kapakata*, *C. salvatrix*, *C. stenophyla*, *C. liberica*, *C. racemosa*, and others are primarily used in genetic crosses. *Coffea arabica*, known as Arabica in the coffee market, makes up 70% of total production volume, and *C. canephora* (Robusta coffee) is responsible for the remaining 30%. Currently, Brazil is the largest producer of arabica coffees, followed by Colombia, Paraguay, Venezuela, Indonesia, Ethiopia, India, Mexico, and 40 other countries. The United States, Brazil, and the European countries are the major consumer markets.

42.2 *Coffea Arabica* and *C. canephora*

Coffea arabica was first described by Linnaeus in 1753. Its fruits are round, smooth, slightly bitter, and chocolaty in color, with a smooth crust and an intense aroma. Brazil is the leading producer and exporter of *Coffea arabica* (ABIC 2010) and the second largest consumer. There are currently 11 varieties most frequently planted in producing countries. These varieties are the result of breeding projects intended to achieve early maturity, a size suitable for mechanical harvesting, rust resistance, high-density planting, and improved beverage quality (Fazuoli 1999).

The *Coffea canephora* species, also known as Robust or Conillon coffee, is stronger and more productive than Arabica coffee, but its flavor is less popular with consumers, and it is mainly used in the formulation of blends and espressos. Indonesia is the largest producer of this coffee, followed by Brazil, Vietnam, the Ivory Coast, and Uganda (Ferreira Júnior and Mitchell 2007).

The two species *Coffea arabica* and *C. canephora* feature differing levels of caffeine, chlorogenic acids, sucrose, and amines (putrescine) (Andueza et al. 2004; Casal et al. 2004, Martin et al. 2001, Gonzalez et al. 2001). Table 1 summarizes the different compounds and their compositions in Arabica and Robusta coffee.

42.3 Postharvest Processing of Coffee Fruits and Beverage Quality

The fruit consists of the peduncle, crown, exocarp (epicarp or crust), mesocarp (mucilage or pulp), endocarp (parchment), spermoderm (testa or outer coat), endosperm (albumen), and embryos with two cotyledons. The coffee bean used for beverage preparation is the endosperm. To obtain green beans, coffee fruits are subjected to three different types of postharvest processing. Figures 42.1, 42.2, and 42.3 shows the three processes—dry, semidry, and wet, respectively—and their different stages. The choice of processing type is subject to the uniformity of fruit maturation, the local weather conditions at harvest time, and the availability of water at the production site. Yield is not a deciding factor because each different process produces equivalent yield. On average, from a 550-lb batch of cherry fruits, 100 lb of processed grains are obtained, which will decrease another 16% in weight after roasting (Hicks 2001).

Convert to metric?

42.3.1 Dry or Natural Process

In general, Robust and Arabic coffees grown in Brazil, Ethiopia, Haiti, Indonesia, and Paraguay are processed using the dry or natural method. This kind of process creates so-called natural or nonwashed coffees. During this process, the intact coffee fruits dry in terraces or platforms of concrete, asphalt, or packed dirt. The process involves few steps and entails low equipment demand (Figure 42.1). The fruit harvesting process, performed via manual stripping, yields fruit lots at different stages of maturation

TABLE 42.1

Chemical Compounds and Their Respective Concentrations Present in Coffee Beans Used as Chemical Markers to Differentiate Between *Coffea arabica* and *Coffea canephora*

Chemical Markers (Dry Weight)	*Coffea arabica*	*Coffea canephora*
Sucrose	9.3	5.45
Putrescine-free (μg/g)	47.9	11.1
Linoleic acid (%)	6.1	3.7
Oleic acid (%)	8.3	12.3
Chlorogenic acids	4.1	11.3
Caffeine (%)	1–2	>3
β-Tocopherol (μg/g)	58.46	13.1

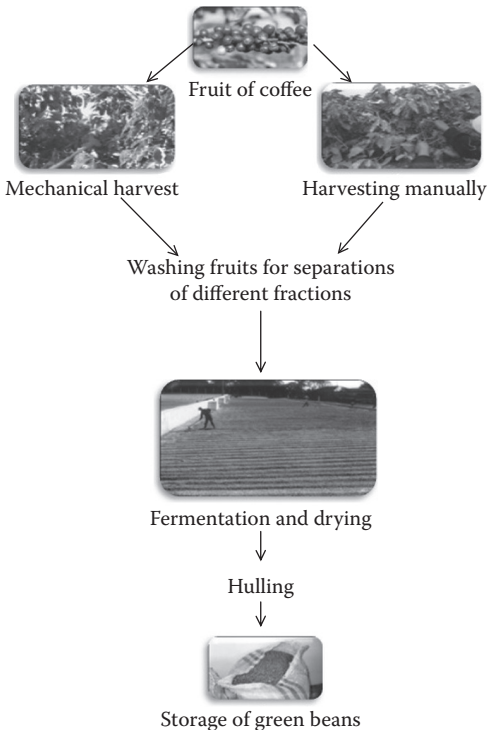

FIGURE 42.1 Main steps involved in dry or natural coffee fermentation.

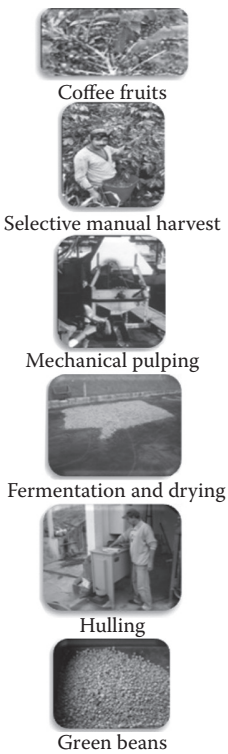

FIGURE 42.2 Main steps involved in wet coffee fermentation.

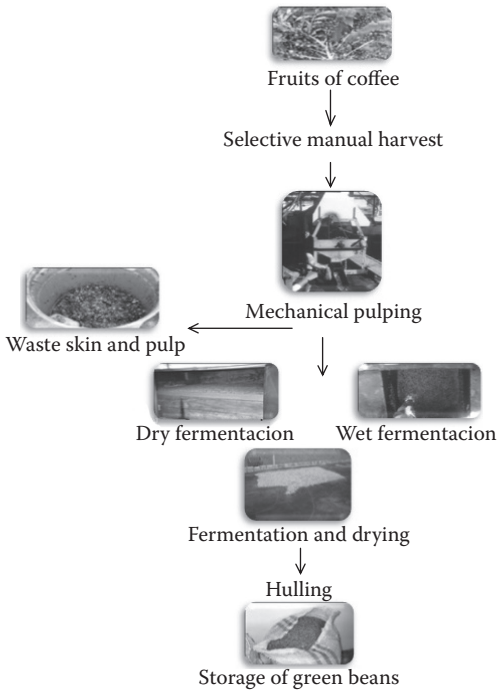

FIGURE 42.3 Main steps involved in semidry coffee fermentation.

(green, yellow green, cherry, dried). These types of fruit are separated by density during the fruit-washing phase. The fruits in the same maturity stage are placed on the threshing floor and remain there for about 15–20 days, depending on weather conditions. The fruits are raked daily so that they will dry evenly, and this process continues until they reach 11% and 12% humidity. This produces the so-called green beans, which are stored in burlap sacks until marketing.

Several physicochemical, microbiological, agricultural, cultural, and processing- and storage-related conditions can influence the final quality of the beverage. Natural coffees are more full-bodied and less acidic than coffees processed using the wet method. The drying and storage of coffee fruit is important to preserving the sensory and sanitary quality. Sanitary quality is preserved by reducing the water content and thus preventing colonization by toxigenic fungi during postprocessing. The drying temperature also affects cell integrity. When the cells of the grains are broken, occur leakage of cellular material, and quality decreases (Borem et al. 2008). Natural fruits cannot be stored above 40°C (Sfredo et al. 2005) if they are to retain maximum quality, and the maximum possible storage time is 90 days (Coradi et al. 2007).

42.3.2 Wet Process

Wet processing gives rise to pulped, peeled, and demucilated coffees, depending on the processing procedure used. Countries such as Colombia, Kenya, countries in Central America and Hawaii perform this type of processing. The steps involved are represented in Figure 42.2 and include the following:

1. Selective harvesting of fruits at the maximum maturity stage (cherry)
2. Mechanical depulping (demucilation)
3. Fermentation in tanks of water and subsequent drying

Roasted green beans will yield beverages that are softer, less viscous, and more acidic (Selmaret al. 2002) than natural coffees. The disadvantage of wet processing is the high consumption of water used to pulp, ferment, and wash beans after fermentation.

The washed coffee beans have a chemical composition different from that of nonwashed beans because the type of processing influences the metabolism of the seeds (Knopp et al. 2006). However, environmental factors and genetic factors related to treatment, agricultural practices, soil and climatic conditions, and technical procedures and postharvest fermentation time can influence the quality of the washed coffee beverage. This is because environmental factors such as shading and altitude influence the formation of aroma precursors such as proteins, amino acids, sucrose, triacylglycerols, chlorogenic acid, and caffeine, as suggested by Vaast et al. (2006). The end of fermentation is determined by the producers based on their tactile perceptions of the fruit. When the fruits are slippery, there is evidence of the presence of mucilage, indicating that fermentation should continue. However, tactile detection may fail, and as a result, Jackels and Jackels (2005) propose the periodic measurement of pH value as an alternative. When this parameter reaches a value of 4.6, this is indicative of the total release of mucilage and thus the end of fermentation.

42.3.3 Semidry Process

The semidry process is a variation on wet processing. It is an intermediate process between dry and wet processing (Figure 42.3) and results in what are called pulped natural coffees (Duarte et al. 2010). Some studies have focused on the chemical properties of coffee beans under semidry processing and the correlation between those properties and beverage quality (Duarte et al. 2010; Tarzia et al. 2010). No study has yet presented conclusive evidence of the level of influence of this process on the final quality of the beverage. However, it is possible to say that the qualities of coffee beverages made from pulped natural coffee lie somewhere between those obtained using dry and wet processing. The green beans produced via semidry processing are usually used in espresso blends (Duarte et al. 2010).

The concentration of aroma precursor compounds such as caffeine, trigonelline, sucrose, and chlorogenic acids can positively or negatively affect flavor. Chlorogenic acids are reported to be responsible for bitterness, and the concentration of these compounds in pulped natural coffees is lower than when wet processing is used. Trigonelline provides a pleasant flavor and is present in lower concentrations in semidry processing than in wet processed grains. Sucrose is the precursor to volatile compounds and corresponds, in dry weight, to 7.5 g/100 g. Aldehydes and carboxylic acids are desirable in high concentrations, as they are responsible for the production of furan. The concentration of sucrose is higher in semidry processing than in wet processing (Selmar et al. 2008).

42.3.4 Storage of Green Beans

After they are processed, the coffee fruits are hulled and stored until they are put on the market. After processing, the factors that affect the preservation of beverage quality are the physical storage conditions and the storage time (Coradi et al. 2007; Borem et al. 2008). Because the grains are stored for up to three years, they should be stored under constant temperature and relative humidity (RH) conditions to prevent the rehydration of the grain and postharvest reactions. The coffee bean embryos remain viable during processing but die after long periods of storage (usually after 6 months). After the death of the embryo, enzymes such as polyphenol oxidase and laccase can remain viable if the grain presents moisture above 20%; these would be inappropriate storage conditions and would allow the rehydration of the grains. The action of PPOs and laccase would cause the oxidation of phenolic compounds and decrease quality (Selmar et al. 2008).

In dry, semidry, and wet coffee processing in Brazil, storage temperatures above 40°C have damaged the cells of the grains, causing the leakage of intracellular material and decreasing beverage quality (Borem et al. 2008; Coradi et al. 2007). Selmar et al. (2008) suggest that the green beans must be stored (RH 63% and 22°C) with parchment because, regardless of the type of processing, the grains are of better quality when they are not hulled.

42.4 Microorganisms Present in Coffee Fruits

Natural or dry processing is mainly used in Brazil, Ethiopia, Haiti, Indonesia, and Paraguay, where there is a low rainfall density during the fruit harvest. Studies focusing on microbial diversity in coffee fruits at different stages of maturity and during the overall drying process have been carried out by Silva et al. (2000, 2008a,b) and Vaughn (1958). These populations are diverse and include bacteria, yeasts, and filamentous fungi, all of which predominate in the fruits as they undergo constant physical and chemical changes. These changes are due to seed metabolism and to the gradual loss of water that occurs on the threshing floor. The drying period corresponds to the fruit fermentation period, in which the pulp and mucilage break down.

The species able to colonize the fruit must also be able to withstand the physicochemical changes that will occur (in pH, sugar content, and moisture), which will secrete pectinases on pectin pulp and mucilage. The depolymerization of pectin is the carbon source for microorganisms after the initial intake of simple sugars. This process is promoted by the 20-day period, during which the fruits remain on the threshing floor, with microbial growth sustained by pectin depolymerization. At the beginning of fermentation, the high water activity (approximately 0.9) facilitates the development and action of bacteria on the coffee fruit that are gradually replaced by yeasts and filamentous fungi at the end of the process (a_w 0.7). The largest bacterial population in the early stages of fermentation was recorded by Vaughn (1958), Silva et al. (2000, 2008a), and the largest population in samples of fresh coffee pulp was reported by Gaime-Perraud et al. (1993).

42.4.1 Bacteria

In natural coffee, some authors (Table 42.2) have identified the presence of Gram-negative and Gram-positive bacteria; in the first days of fermentation, the average population found is 10^6–10^9 CFU/g. Along with the high bacteria count in natural coffee fruit, there is also a great deal of species diversity, some native to the fruit, and so originating from the soil, agricultural tools, air, and water (Silva et al. 2008a). Thus, some species present in the fruit may not be part of the fermentation process.

All species identified in coffee fruits at different stages of natural maturation and processing are listed in the studies by Silva et al. (2000, 2008a,b). Among these species are *Tatumella ptyseos*, *Pseudomonas putrefaciens*, *P. mirabilis*, *Enterobacter aerogenes*, *Acinetobacter*, and *B. subtills* (Silva et al. 2000, 2008a), and *Paenibacillus amylolyticus* (Sakiyama et al. 2001), which is an endophytic species that presents pectin lyase activity and is therefore co-responsible for the fermentation of natural coffee, along with some yeast species.

TABLE 42.2

Bacteria Species Identified During the Period of Natural (Dry) Fermentation Coffee Fruits

Reference	Country	Bacteria
Silva et al. (2000, 2008b) Sakiyama et al. (2001) Vaughn (1958)	Brazil	Gram-positive: *Bacillus cereus*, *B. subtilis*, *B. macerans*, *B. megaterium*, *B. stearothermophilus*, *B. laterosporus*, *Cellulomonas. Arthrobacter*, *Microbacterium*, *Brochothrix*, *Dermabacter*, *Lactobacillus*, *Acinetobacter* sp., *B. polymyxa*, *Kurthia*, *Paenibacillus amylolyticus* Gram-negative: *Aeromonas*, *Enterobacter agglomerans*, *Pseudomonas*, *Serratia rubidea*, *S. plymutica*, *Hafnia*, *Tatumella ptyseos*, *Flavobacteriu*, *Klebsiella*, *Chromobacterium*, *Pasteurella*, *Acinetobacter*, *Cedecea*, *Citrobacter*, *Shigella*, *Providencia mirabilis*
De Bruyne et al. (2007)	Ethiopia	*Leuconostoc*
Van Pee and Castelein (1972)	Congo	*Erwinia dissolvens*, *Hafnia*, *Enterobacter aerogenes*, *E. cloacae*, *Klebsiella*

The presence of organic acid from fermentation metabolism (acetic, lactic, butyric, and propionic acids) confirms the microbial action in the fermentation process for natural coffees. In a study by Silva et al. (2008a), the production of organic acids from microbial metabolism and seed via high-performance liquid chromatography was assessed. In that study, it was observed that butyric acid is not detected in any of the samples analyzed. The presence of this acid in coffee beans generally gives an unpleasant flavor to the beverage and thus leads to quality loss. Acetic acid is detected mainly in the pulp and in parts of the mucilage. Acetic acid production is an aerobic metabolic process that can be of bacterial origin or the product of the oxidation of yeast-produced alcohol. The bacteria present on the surface of the coffee cherries synthesize the acid that can migrate to the pulp and mucilage and can interfere with the organoleptic quality of the beans. The negative influence of bacteria on the quality of the coffee beverage is linked to the species present and not to population density. In natural coffee, ruit rated very soft (i.e., as having optimal sensory quality), the bacterial population can represent up to 80% of total microbiota during this process.

42.4.2 Yeasts

The yeast population in natural coffee is usually lower than the bacterial population, presenting average values of 10^4 CFU/g; it becomes predominant with the reduction of water activity during the drying period and is detected in fruits with a_w of 0.6. *Pichia*, *Candida*, and *Arxula* are the most commonly found genera in fermented and dried fruits (Silva et al. 2000, 2008a). The species identified by Silva et al. (2000, 2008a) on the surface of coffee fruits included *Arxula adeninivorans*, *Blastobotrys proliferans*, *Candida auringiensis*, *C. glucosophila*, *C. incommunis*, *C. membranifaciens*, *C. paludigena*, *C. schatarii*, *C. saitoana*, *Candida fermentati*, *C. vartiovaarae*, *Citeromyces matritensis*, *Debaryomyces polymorphus*, *D. hansenii*, *Geotrichum fermentans*, *Pichia guilliermondii*, *Pichia acaciae*, *P. anomala*, *P. burtonii*, *P. ciferii*, *P. jadinii*, *P. lynferdii*, *P. ofunaensis*, *P. sydowiorium*, *P. subpelliculosa*, *Saccharomyces cerevisiae*, *Saccharomycopsis fermentans*, *S. fibuligera*, *Schizosaccharomyces cpombe*, *Sporopachydermia cereana*, *Stephanoascus smithiae*, *Trichosporonoides oedocephales*, and *Williopsis saturnus* var. *sargentensis.*

The involvement of yeast species in the fermentation process and bacterial action can be assumed based on their potential to produce pectin lyase and polygalacturonases. Of the identified species, *Debaryomyces hansenii*, *Pichia sydowiorium*, *Sthephanoascus smithiae*, and *Arxula adeninivorans* are those that have the capacity for pectin lyase production and that therefore cause degradation of the pulp and mucilage of natural coffee fruit (fermentation) via pectinolytic bacteria). The role of yeast may be related not just to the fermentation process but also to the control of filamentous fungi growth. Isolates belonging to the genera *Debaryomyces* and *Pichia* have demonstrated the ability to inhibit the growth of toxigenic fungi and may therefore have the potential for biological control (Ramos et al. 2010).

42.4.3 Filamentous Fungi

Studies of natural coffee microbial populations always emphasize filamentous fungi isolation and identification, but the microorganisms present during the fermentation and drying period are predominantly bacteria and yeasts. Therefore, the presence of filamentous fungi is not involved in the fermentation process; these are detected in the last days of fermentation and during storage. Various levels of fungal and toxin levels detected in processed dry beans can be found in studies by Batista et al. (2009), Batista and Chalfoun (2007), Silva et al. (2003), De Moraes and Luchese (2003), Chalfoun and Batista (2007), and Taniwaki et al. (2003).

The incidence of fungi in coffee fruits and beans is associated with a decrease in the sensory quality of the final coffee beverage, probably due to the production of metabolites such as superior acids. One exception is the presence of *Cladosporium cladosporioides*, which is usually found in high-quality beverages (Pereira et al. 2005; Chalfoun et al. 2007).

The species of filamentous fungi isolated in natural coffee are *Alternaria*, *Aspergillus flavus*, *A. niger*, *A. tamarii*, *A. ochraceus*, *A. wentii*, *A. versicolor*, *A. glaucus group Cladosporium herbarum*, *Fusarium stilboides*, *Penicillium chrysogenum*, *P. viridicatum*, *P. islandicum* (Mislivec et al. 1983—countries of

Central and South America, Asia, and Africa), *Aspergillus flavus*, *A. fumigatus*, *A. niger*, *A. parasiticus*, *A. sydowii*, *A. tamarii*, *A. terreus*, *Cladosporium cladosporioides*, *C. herbarum*, *C. macrocarpum*, *Fusarium oxysporum*, *Penicillium albidum*, *P. brevicompactum*, *P. chrysogenum*, *P. citrinum*, *P. cyclopium*, *P. funiculosum*, *P. jensenii* (Abdel-Hafez and El Maghraby 1992—Egypt), *Aspergillus fumigatus*, *A. niger*, *Fusarium graminearum*, *Penicillium granulatum* (Liardon et al. 1992—Brazil), *Mucor*, *Cercospora*, *Phoma*, *Penicillium* (Alves and Castro 1998—Brazil), *A. carbonarius*, *A. niger*, *A. ochraceus*, *Penicillium* sp. (Taniwaki et al. 2003—Brazil), *A. ochraceus*, *Aspergillus* section *Nigri*, *Rhizopus* (Pardo et al. 2004—Angola, Bolivia, Brazil, Costa Rica, Ecuador, Ethiopia, Guatemala, India, Indonesia, Ivory Coast, Java, Nicaragua, Uganda, and Vietnam), *A. tubingensis*, *A. carbonarius*, *A. ochraceus*, *A. sulphureus*, *A. tamari*, *A. niger*, *A. flavus* (Magnani et al. 2005—Brazil), *Cladosporium cladosporioides* (Chalfoun et al. 2007—Brazil), *A. ochraceus*, *A. flavus*, *A. sulphureus*, *A. niger*, *A. versicolor*, *A. sydowii*, *A. ostianus*, *A. melleus*, *A. dimorphicus*, *A. sclerotiorum*, *A. auricomus*, *A. foetidius*, *Eurotium amstelodami*, and *E. chevalieri* (Batista et al. 2001, 2007, 2009—Brazil), *A. flavus*, *A. niger*, *A. ochraceus*, *A. tamari*, *A. sydowii*, *A. foetidius*, *A. dimorphicus*, *Fusarium lateritium*, *F. solani*, *F. illudens*, *F. stilboides*, *F. semitectum*, *P. brevicompactum*, *P. crustosum*, *P. roqueforti*, *P. citrinum*, *Cladosporium cladosporioides*, *Paecelomyces* sp. (Silva et al. 2000, 2008a,b—Brazil).

42.4.3.1 Ochratoxin A–Producing Toxigenic Fungi

The toxigenic potential of a fungus is defined as the ability of a strain to produce toxic metabolites. Mycotoxins are characterized as secondary metabolites and are produced between the late exponential phase and early stationary phase of fungal growth; they are therefore not related to the biological role of microorganisms (Bars and Bars 2000). The conditions that enable the production of mycotoxins by a potentially toxigenic strain are more limited than those that permit the growth of fungi. Many strains of a species that is considered toxigenic may not possess this property. In potentially toxigenic species, the production of toxins can vary by up to 1000 times (Bars and Bars 2000).

Ochratoxin A present in coffee samples has mainly been attributed to the presence of species of the genus *Aspergillus* belonging to section *Circumdati* and section *Nigri* (Urban et al. 2001; Batista et al. 2003). Ochratoxin A is a secondary metabolite that is mainly produced by fungi belonging to the genera *Aspergillus*, *Petromyces*, *Neopetromyces*, and *Penicillium* (Frisvad and Samson 2000), but the genus *Aspergillus* is undoubtedly one of the most important for coffee. Among the species of the genus *Aspergillus* belonging to section *Circumdati*, the leading producer of ochratoxin A is *A. ochraceus*, but other species belonging to this section and producing ochratoxin A have been found in coffee fruit and beans, including *A. elegans*, *A. auricomus*, *A. ostianus*, *A. petrakii*, *A.*, and *A. sclerotiorum sulphureus* (Batista et al. 2003). Frisvad et al. (2004) and Sartori et al. (2006) indicated that *A. westerdijkiae* can also contribute to the presence of ochratoxin A in coffee beans in Brazil. Studies have revealed that *A. carbonarius* is more common and represents the major source of ochratoxin A in section *Nigri*, whereas *A. niger* is rarely the producer (Heenan et al. 1998). Others studies indicate that other species including *A. steynii*, *A. westerdijkiae*, *A. lactofeatus*, and *A. slerotioniger* (Frisvad et al. 2004; Samson et al. 2004) can also be producers of ochratoxin A in coffee. In the genus *Penicillium*, the only species considered producers of ochratoxin A are *Penicillium verrucosum* and *P. nordicum* (Larsen et al. 2001).

Missing species here.

Both the environmental conditions of the geographic area and the types of grains influence the population of fungi and mycotoxin production. The study of the economic importance of *Aspergillus* species using polyphasic taxonomy is an innovative strategy that assists in characterizing the biodiversity of species and evaluating the risk of ochratoxin A contamination in coffee beans.

42.5 Microbiota of Wet Processed, Depulped, and Washed Coffee

The mechanical removal of pulp that occurs in wet processing allows mucilage to remain adhered to coffee beans. This mucilage is the substrate used by bacteria and yeast during fermentation. During fermentation, the precursors of flavor-enhancing compounds are naturally excreted by the fermentative microbiota present in coffee fruits (Gonzalez-Rios et al. 2007).

The microbial diversity of coffee beans in wet processing is lower than in dry processing due to fermentation conditions like reduced fermentation time (48 hours), faster decreases in pH, and pulp removal. The microbiota includes few species of bacteria and yeast and have been no reports of filamentous fungi involved in the fermentation process. The greatest microbial diversity in washed coffee fruits can be recognized using molecular techniques that allow the detection of culturable and unculturable microorganisms. The molecular identification of microorganisms associated with coffee has been performed by Vilela et al. (2010) for semidry processing and Masoud et al. (2004) for wet processing. In semidry processing, microbial succession occurs, bacteria dominated at the beginning of the process, but after 72 hours, bacteria and yeasts populations reach similar values (average 10^6 CFU/g), and the yeast dominate after 192 hours of fermentation (Vilela et al. 2010). This process is similar to the process of ecological succession described in Silva et al. (2008a), who observed that filamentous fungi were rarely found in semidry processes.

42.5.1 Bacteria

Studies dating back to the 1920s have isolated bacteria, fungi, and yeasts from coffee fruits, but do not make inferences about the involvement of microorganisms in mucilage degradation. Currently, there is still doubt about the apparent involvement of bacteria in coffee fruit fermentation, and since 2002, no results that address this issue have been published. An early study on the action of microorganisms present in coffee fermentation was published in 1946 by Pederson and Breed (1946) using samples of coffee from Colombia and Mexico. These authors isolated cocci and microaerophilic bacteria such as *Leuconostoc mesenteroides*, *Lactobacillus plantarum*, *Lactobacillus brevis*, and *Streptococcus faecalis* as facilitating the lysis of mucilage but not its detachment. Agate and Bhat (1966) isolate and identify *Streptococcus*, *Pseudomonas*, *Flavobacterium*, and *Proteus* from depulped coffees in India and state that these bacteria are not pectinolytic and are therefore not involved in the process of fermentation of depulped fruits.

Avallone et al. (1999, 2001, 2002) have isolated bacteria from wet processed fruits and detect the Gram-negative bacteria *Erwinia* and *Klebsiella* in a culture containing pectin, isolated from the coffee. They were characterized by low pectinolytic capacity and produced pectate lyase. The authors support the hypothesis that the presence of bacteria is facilitated by the consumption of simple sugars because the production of pectate lyase keeps these bacteria from affecting the fruit mucilage; the bacteria that produce polygalacturonase are rarely identified as *Lactobacillus brevis*. Microorganisms that facilitate the degradation of mucilage must be capable of secreting pectin lyase and polygalacturonase due to the methylated structure of pectin.

In semidry processing, 15 species, including *Acinetobacter*, *Bacillus cereus*, *B. macerans*, *B. megaterium*, *B. subtilis*, *Enterobacter agglomerans*, *Erwinia herbicola*, *Escherichia coli*, *Klebsiella pneumoniae*, *Lactobacillus brevis*, *L. plantarum*, *Lactococcus lactis*, *Leuconostoc mesenteroides*, and *Serratia* (Vilela et al. 2010), have been identified using traditional and molecular methods. Some of these species have also been identified in coffee cherries, possibly due to natural, common aspects of these two processes.

42.5.2 Yeasts

The population of yeasts in depulped coffees is greater than that of bacteria at the beginning of the fermentation period, with a density close to 10^4 CFU/g that increases during fermentation, reaching a value of 10^7 CFU/g (Masoud et al. 2004).

Of the studies that have been published on the isolation and the role of microorganisms in the fermentation of depulped coffee, only the research by Avallone et al. (2001, 2002) has not inferred the degradation of mucilage to be a product of the action of bacteria and/or yeast, which are responsible for the production of ethanol in overfermented coffees. However, pectinolytic yeasts have been isolated during the wet processing of coffee (Masoud and Jespersen 2006; Agate and Bhat 1966; Daivasikamani and Kannan 1986; Van Pee and Castelein 1971) in both Robust and Arabic coffees.

Agate and Bhat (1966) observed that the yeasts found in Robust coffee beans from India are predominant over the population of bacteria. The yeasts *Kluyveromyces*, *Saccharomyces* sp., *Saccharomyces marxianus [Kluyveromyces marxianus* (EC Hansen) van der Walt (1971)], *S. bayanus*, *S. cerevisiae*

var. *ellipsoideus* produce pectinases that degrade mucilage pectin. In that instance, it was impossible to confirm the degradation of mucilage because the degradation of mucilage was not observed when the depulped fruits were sterilized. Working with depulped coffees from the Congo, Van Pee and Castelein (1971) have identified yeasts of the genus *Candida*, with *C. guilliermondii* var. *membranaefaciens* found on the surface and mucilage of the grains; *C. parapsilosis*, *Saccharomyces cerevisiae*, *Torulopsis famata* [*Candida famata* (Harrison) SA Meyer & Yarrow (Yarrow and Meyer 1978)], *Saccharomyces marxianus* [*Kluyveromyces marxianus* (EC Hansen) van der Walt (1971)], *Candida tropicalis*, *Rhodotorula mucilaginosa*, and *Candida pelliculosa* were found on the surface of grains.

The different types of pectinase produced by organisms can affect the degree of depolymerization of the pectin components, which can then influence the selection of the dominant group of microorganisms (Jones and Jones 1984). The depolymerization of pectin can be performed using the enzymes pectin lyase, pectin methyl esterase, and polygalacturonase. Pectin from the fruit mucilage will be degraded via pectin lyase and polygalacturonase.

The presence of *Kluyveromyces marxianus* in wastewater for fermentation tanks and of *Pichia kluyvery*, *P. anomala*, *Hanseniaspora uvarum* with high pectinolytic capacity seems to enhance the action of yeast during the fermentation process (Serrat et al. 2002; Masoud and Jespersen 2006). The latter three species are capable of producing polygalacturonase in vitro under physicochemical conditions (pH 5.3–3.5 and 30°C) similar to those characteristic of the fermentation process. Thus, the authors conclude that yeasts use mucilage pectin as a carbon source with simple sugars (glucose and fructose), inducing polygalacturonase production.

Similarly, it has been observed that *Pichia kluyvery*, *P. anomala*, *Hanseniaspora uvarum* (Masoud and Kaltoft 2006) have an inhibitory effect on the growth of *Aspergillus ochraceus*, a fungus that is potentially toxigenic and is often isolated in coffee fruits and beans (Batista et al. 2009; Silva et al. 2008a,b). Thus, yeasts play a dual role during the processing of coffee fruits: they facilitate fermentation and biological control.

Species identified in washed and natural coffee fruits have also been found in semidry processing (Vilela et al. 2010). *Pichia anomala* have been shown to be similarly present in freshly harvested fruits (10^4 CFU/g) and have persisted through the 216-hour-long fermentation process. The persistence of this species may suggest the involvement of yeast in mucilage degradation. Other identified species include *Arxula* sp., *Candida ernobii*, *C. fukuyamaensis*, *C. membranifaciens*, *C. carpophila*, *Hanseniaspora uvarum*, *Kloeckera*, *Kluyveromyces* sp., *Pichia caribbica*, *Rhodotorula mucilaginosa*, *Saccharomyces bayanus*, and *Torulaspora delbrueckii*.

42.6 Final Considerations

Coffee is among the most consumed nonalcoholic beverages worldwide, and its production has significant financial ramifications in producing, exporting, and importing countries. The species *Coffea arabica* and *Coffea canephora* are the most used due to their cultural and sensory characteristics. Various chemical compounds are used to certify the coffee species used and the types of processing employed, as the beans have an aggregate value that is directly dependent on their quality. After harvesting, the fruits are processed via dry, semidry, and wet processing, which create different aromas and flavors, and it is up to the consumer to choose the type of beverage that he or she best likes. During processing, the fruits undergo a microbial fermentation process that is responsible for the spontaneous degradation of pulp and fruit mucilage. The microbiota involved in the fermentation process includes several species of bacteria, yeasts, and filamentous fungi. These seem not to participate in the fermentation process and are usually related to decreased beverage quality, with the exception of the *Cladosporium* species. The presence of filamentous fungi may create the risk of contamination by mycotoxins for fruits and coffee beans. The adoption of good agricultural practices and the hazard analysis and critical control points system will significantly reduce the risk of contamination by microorganisms and decrease the incidence of conditions in which the deterioration of the fruits or coffee beans may occur, for instance, by reducing ochratoxin A.

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