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Review

Challengs in coffee quality: Cultural, chemical and microbiological aspects

Desafios na qualidade de café: Aspectos cultural, químico e microbiológico

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ABSTRACT
The Brazilian coffee industry is undergoing a great transformation in order to serve a consumer market that is becoming increasingly demanding with regard to quality. Considering the multiple determinants of the final quality of the product, one must consider factors that are involved in steps from the pre-harvest stage to storage. The execution of the different stages according to good-practice programmes has repercussions on microbiological, physical and chemical characteristics, which in turn affect the quality of the final product with regard to sensorial properties and safety. There has been research progress in the improvement of quality evaluation techniques that minimize the subjective effects of traditional classification. It is also observed that socio-environmental aspects of coffee production, while not the subject of this review, have broadened the concept of quality since an increasing number of consumers are interested in aspects regarding agricultural sustainability in addition to strictly sensorial aspects.

Index terms: Café; classificação; avaliação de qualidade.

RESUMO
A cafeicultura brasileira passa por uma profunda transformação visando atender um mercado consumidor cada vez mais exigente quanto a qualidade. Considerando-se os múltiplos fatores determinantes da qualidade final do produto, há que se considerar fatores que envolvem as etapas desde a pré- colheita até o armazenamento. A execução das diferentes etapas segundo programas de Boas Práticas apresenta repercussão sobre as características microbiológicas, físicas e químicas traduzidas pela qualidade do produto final quanto às características sensoriais e de segurança. A pesquisa tem sido progressista na busca de aprimoramento das técnicas de avaliação da qualidade visando minimizar os efeitos subjetivos das técnicas de classificação tradicionais. Observa-se ainda que aspectos socioambientais da atividade, apesar de não ser objeto da presente revisão, tem ampliado o escopo do conceito de qualidade uma vez que um número crescente de consumidores estão interessados em aspectos quanto a sustentabilidade da cultura, além do aspecto estritamente sensorial.

Termos para indexação: Coffee; ranking; quality assessment.

INTRODUCTION

Coffee production conditions, as well as post-harvest operations, such as fruit selection, processing, drying and storage conditions, can influence the quality of coffee beans (Clemente et al., 2015).

The physical characteristics and chemical composition of coffee are influenced by a variety of factors, including genetic, environmental, and nutritional factors, and crop management, harvesting, and preparation. With the exception of the genetic and environmental factors, these factors can be controlled, not only via field management but also by adopting good agricultural practices, after crop implantation, when all the effort goes towards maximizing quality, and in the coffee harvesting phase, during which the preservation of quality is sought (Mesquita et al., 2016).

The present review addresses not only the importance of the proper execution of each stage, pre-harvest, harvest and processing, but also discusses how this execution can be adequately. Quality, in this article, is the quality of the coffee sensu stricto, referring to the physical, chemical and microbiological aspects related to the final quality of the beverage.

This review also discusses the efforts to improve the methodologies used to categorize products by quality, minimizing subjective factors that may reduce the reliability of results.

However, for a holistic view, it is important to consider issues related to crop sustainability, covering social, economic and environmental aspects of coffee production. Sustainability is part of the demands of a growing number of consumers and should be considered, given that coffee quality is mainly defined to meet consumer demands.

PRE-HARVEST, HARVEST AND COFFEE-BEAN QUALITY

Production conditions and post-harvest operations, such as fruit selection, processing, drying and storage, can influence the quality of coffee beans (Clemente et al., 2015).

The physical characteristics and the chemical composition of coffee are influenced by a variety of factors, including genetic, environmental, and nutritional factors, and crop management, harvesting, and preparation. With the exception of the genetic and environmental factors, these factors can be controlled not only by management (good agronomic practices) after crop implantation , when all the effort goes towards maximizing quality, but also in the coffee harvesting phase, during which the preservation of quality is sought (Mesquita et al., 2016).

Pre-harvest

Borém (2008), warn that good harvest planning starts with the clearing of loose soil as well as leaves, weeds, fruits remaining from the previous harvest, and other debris.

This phase, which is of fundamental importance in the management of coffee production, consists of forecasting time and duration, designing the method of collection, collecting materials and financial resources; and designing and refining the infrastructure and machinery for the processing of each type of final product to be obtained (pulp, peeled cherry, etc.) (Mesquita et al., 2016). Techniques such as weeding and weed control as well as the use of foliar sprays based on fungicides or products that prevent the appearance of pathogens are important measures during the harvest since these techniques guarantee sanitary conditions as well as efficiency during harvesting (Ameyu et al., 2017).

It is imperative that the need for cultivation is met by using essential agricultural practices that influence production and productivity, such as the use of suitable cultivars for the region, pest and disease control, soil repair, fertilization and mineral nutrition (Oliveira; Oliveira; Moura, 2012).

Harvest

In Brazil, the coffee harvest period generally begins in May and lasts until August. Factors such as climate and the amount of coffee on the plant or fallen on the ground interfere with the beginning and end of processing (Ventureli et al., 2016).

The application of appropriate harvesting techniques is also an extremely important factor for producers as it contributes to higher economic returns. According to Silva, Salvador and Padua (2012), there are three types of harvesting techniques for coffee: manual, which is carried out manually and requires a large amount of labour; semi-mechanized, in which both manual and mechanized harvesting operations are used; and mechanized, in which all harvesting operations are performed mechanically. The type of crop used may vary according to local topography and incline and the spacing, alignment and height of the plants (Ventureli et al., 2016); thus, based on these factors, producers should choose the type of harvest that best suits their crop.

It has been observed that fruit in the cherry stage exhibit better drinking patterns as this phase corresponds to the ideal point of maturation of the fruit, in which the bark, pulp and seed have a suitable chemical composition, which leads to fruit of the highest quality. These fruit contain volatile compounds that are responsible for the characteristic flavour and aroma of coffee and are present at very low sensory values in green fruit, the values increasing gradually with maturation, with cherry fruit assuming optimal values (Pimenta et al., 2008).

The best type of beverage is produced from coffee cherries, provided that the fruit is properly processed; however, because crop concentration occurs at the same time, some growers tend to anticipate the coffee cherries when the percentage of green fruit is 5% higher than the recommended value (Chalfoun; Azarias; Martins, 2016). In addition, in the cherry stage, the coffee fruit presents a smaller detachment force, which facilitates harvesting (Silva et al., 2013; Santinato et al., 2015).

Obtaining high maturation uniformity is a major challenge in the coffee sector and is a procedure that provides consistent quality. Matching the species/cultivar to the best cultivation site followed by appropriate agricultural treatment has been shown to be decisive in the reduction of unevenness in maturation uniformity. It is important to note that the climatic conditions in year of growth can change the number of flowering plants, which will directly affect the desired maturation uniformity (Pimenta et al., 2008).

The determination of the best time to harvest a majority of the fruits in the cherry stage, along with other factors, is essential for obtaining a coffee with adequate chemical composition and minor undesirable chemical modifications and detriments to the quality of the product. The time of harvest is essential as it is directly related to the various physical, physicochemical and chemical constituents that are responsible for the appearance of the roasted bean and flavour and aroma of the drinks; among these compounds are volatile constituents, phenolics (chlorogenic acid), fatty acids, proteins and some enzymes whose presence, concentrations and activities confer a unique flavour and aroma to the coffee (Amorin; Amorin, 1977; Angélico et al., 2011; Ribeiro et al., 2014; Duran et al., 2017). Selective harvesting, coffee separation and well-conducted drying can produce high-quality coffee (Arruda et al., 2011).

COFFEE PROCESSING

Processing and drying are important phases in the post-harvest phase of coffee production because these processes directly influence the final quality of the product. After harvesting, the fruits can be processed by two different methods: the dry method and the wet method. An additional mode of processing is known as semi-dry processing, a variation of the wet process (Malta; Chagas, 2010; Giomo, 2012; Duran et al., 2017).

The choice of the method of coffee processing greatly impacts the profitability of coffee production and depends on several factors, such as climatic conditions; capital availability; technology and equipment; consumer-market requirements regarding product characteristics; water availability; and technology for the treatment of wastewater (Malta, 2011).

Regardless of the process used, after harvesting, the coffee fruit must be processed and spread in the shortest possible time. The fruit must never be crowded or remain in the carts, waiting for discharge, since humidity and temperature conditions in the coffee mass are highly favourable to the development of microorganisms that accelerate the fermentation process (Pimenta et al., 2008; Angélico, 2011).

In the dry preparation, the coffee cherries are completely dried in yards or on patios, with pre-dryers or mechanical dryers, without the removal of the bark, giving rise to coconut coffee. While this type of preparation is the most commonly used for Brazilian coffee, wet preparation has been considered a viable alternative to obtain coffee of higher quality (Alves et al., 2013; Nogueira; Roberto; Sampaio, 2017).

Wet preparation is the process by which, after passage through washers, the exocarp and/or mucilage of the fruit is removed, reducing the risk of fermentation and allowing faster drying, which generally results in good quality (Malta, 2011). Wet preparation is a common practice among producers in Mexico, Colombia and Kenya, but in Brazil, a little pulp is still used. This method is recommended for areas where the post-harvest period occurs under conditions of high relative humidity (Nogueira; Roberto; Sampaio, 2014).

Traditionally, pulping is carried out via spontaneous fermentation in concrete tanks, removing the remaining mucilage adhered to the parchment as it is a suitable substrate for the development of microorganisms that can cause fermentation, which is detrimental to the final quality of the product. The coffee remains in these tanks with water for a period of 12 to 36 hours to eliminate the mucilage. After this period, the beans are washed until no sign of this mucilage is detected, and then the beans are dried (Malta, 2011; Chalfoun; Fernandes, 2013; Nogueira; Roberto; Sampaio, 2014).

In the semi-wet process, the ripe fruits are mechanically husked, and part of the mucilage remains adhered to the fruit parchment. This operation is performed on husking machines based on the difference in resistance to pressure of green fruit and ripe fruit (Malta, 2011).

After pulping, demucilation is carried out in demucilating machines in order to remove the mucilage that remains adhered to the beans. The removal is mechanical and occurs due to the friction between beans and between beans and the metal cylinder. In this apparatus, water is added in small quantities for lubrication and cleaning of the mucilage (Malta, 2011). The main advantage of using this equipment is the removal of part of the mucilage without using fermentation tanks; besides facilitating the work of movement and drying in the yard, by this method, the beans do not form agglomerates as they do in the pulped natural cherry process (Alves, et al., 2013).

DRYING

Coffee drying is one of the most important post-harvest practices. This process is aimed at reducing the water content of the product, consequently reducing the risk of infestation by microorganisms, reducing the occurrence of enzymatic fermentation, preserving quality and nutritive value, and ensuring germinative power. The drying process can be classified into two types: natural drying and artificial drying (Donzeles et al., 2011; Taveira et al., 2015).

This operation is more difficult to perform compared to other processes because of the high moisture content (60% to 70%) of the fruit at the time of harvest (Donzeles et al., 2011; Ghosh; Venkatachalapathy, 2014). In Brazil, three types of drying processes are used: drying in yards, mechanical dryers and combined drying (Ferreira et al., 2013).

Regardless of the drying method used, the following points should be emphasized for successful post-harvest processing of coffee: to prevent the coffee from fermenting before and during drying, avoid excessively high temperatures during drying, since coffee can tolerate air temperatures of approximately 40 °C for one or two days, 50 °C for a few hours and 60 °C for less than an hour during drying without damage; dry the beans up to 18% bu in the shortest possible time; and seek to obtain a uniform coloration of the product (Donzeles et al., 2011).

PROCESSING AND STORAGE

Processing is an operation carried out after the drying of the coffee and constitutes the separation of beans, bark and impurities by peeling the coconut coffee and separating the bark and parchment, thereby eliminating most of the impurities. This procedure must be conducted correctly and carefully, otherwise impurities remain in the middle of the beans processed which is considered a defect and adversely affects the classification of the coffee by type (Silva; Alves, 2013).

Re-sorting is increasingly being used, mainly by cooperatives and companies that market coffee. This process is adopted for the purpose of classifying the coffee and improving quality by eliminating defects. This process is based on bean size, shape, specific gravity, magnetic properties and colour.

Coffee beans are conventionally stored in jute bags that have the capacity to hold 60 kg of processed coffee. Storage in big bags, which have the capacity to hold 1,200 kg of coffee, has the advantages of adaptation to mechanized handling, reduction of losses associated with the loading and unloading of the coffee, and reduction of the labour required. An additional way to store coffee is bulk storage, which does not use bags, with the coffee being stored in silos or bins (Borém, 2014).

CLASSIFICATION OF COFFEE IN BRAZIL

The classification of coffee involves the separation of beans into categories, subcategories, groups, subgroups, classes and type according to sensorial characteristics. At the category level, coffee is classified as *Coffea arabica* L. or *Coffea canephora* P., and at the subcategory level, coffee is classified as flat beans (with normal development) or mocha beans (beans with ovoid shape due to the lack of fertilization of one of the eggs), which are sorted by different kinds of sieves: round screen for flat beans and oblong screen for moca beans. When the coffee cannot be sorted by sieves or when the beans fall through four or more sieves, the coffee obtained is called Bica Corrida (Brasil, 2003).

Based on aroma and flavour, the raw coffee beans are classified into two groups, as determined by means of a cup test: Group I (Arabica) and Group II (Robusta). The coffee is further classified into subgroups. The subgroups of Arabica are as follows: Strictly soft: coffee that, overall, presents all the requirements of aroma and “soft” flavour but is more accentuated; Mole: coffee that has a pleasant and sweet aroma and taste; Mole only: coffee that has a slightly sweet and smooth flavour but without astringency or roughness; and Hard: coffee that has an acrid, astringent and rough taste but does not have a strange flavour. Additional phenic beverages include the following: Rioy: coffee that has a light taste, typical of iodofórm; Rio: coffee that presents the typical and accentuated taste of iodofórmio; Rio Zone: coffee that has a distinct aroma and taste that is similar to iodoform or phenoic acid, which is disgusting to the palate. The classification of the beverages of Group II (Robusta) are as follows: Excellent: coffee that has a neutral flavour and medium acidity; Good: coffee that has a neutral flavour and slight acidity; Regular: coffee that presents a typical robust flavour without acidity; and Abnormal: coffee that confers a non-characteristic flavour to the product (Brasil, 2003).

In addition, the beans can be classified according to coloration, which varies based on factors such as drying process, air exposure, storage, damage suffered during pulping and processing. Based on colour, the coffee can be classified into eight types: Bluish-Green and Green-Cane: characteristic colours of pulped or degummed coffee; Green: coffee that presents beans that are various shades of green; Yellowish: coffee that presents yellowish beans, indicating signs of ageing of the product; Yellow; Brown; Chumbado; Whitish; Mixture: a mixture of different combinations of colours (Brasil, 2003).

The classification of quality based on appearance, type, and classification of the beverage by the cup test can be complemented by adopting physical, chemical and physicochemical methods that would facilitate the evaluation, making the evaluation less subjective (Silva et al., 2008).

Statistical studies have cast doubt on the accuracy with which tasters classify coffee based on the quality of the beverage. Thus, new parameters, such as physical, physicochemical and chemical characteristics, should increasingly be studied and understood by researchers and combined with software development to validate the conventional cup test, as is the case with the classification systems presented by Silva et al. (2013), which are based on some chemical and physicochemical quality indicators of Class Tor roasted coffee and Class Café raw coffee, using software that has already been validated and will be made commercially available.

SPECIALTY COFFEES

Currently, the specialty coffee sector represents approximately 12% of the international market for the drink and 15% of the market in Brazil. There are a wide range of quality-related attributes of coffee, ranging from physical characteristics, such as origin, varieties, colour and size; to environmental and social concerns, such as production systems and working conditions of the labour force in the coffee industry. Specialty coffees are currently priced higher than conventionally grown coffee by 30% to 40%, exceeding the 100% barrier in some cases. To distinguish specialty coffees, physical and sensorial attributes such as the quality of the drink must be based on the quality of the beverage, which must be higher than the standard. The main categories of specialty coffees are as follows: coffee of certified origin (which is related to the regions of the plantations of origin, as some of the quality-related attributes of the product are inherent to the region where the plant is grown), gourmet coffee (Arabica coffee beans with a sieve of more than 16 and a high-quality, specialty product that is almost free of defects), organic coffee (produced by following the rules of organic agriculture) and fair trade coffee (coffee consumed by those concerned with the social and environmental conditions under which coffee is grown). In this case, the consumer pays more for coffee produced by small farmers or protected production systems, where the crop is associated with the forest (BSCA, 2016).

Specialty coffees are obtained from defect-free beans (black, green, burnt and green-black), with the final beverage being clean, i.e., with no undesirable defects, fermentation or bitter taste, acquiring a pleasant aroma, flavour and long-lasting aftertaste (SCA, 2017). Specialty coffees are those that do not present primary defects such as sticks and stones and present flavours that differentiate these coffees from other beverages, such as floral, citrus, and chocolate flavours, adding value to the product (BSCA, 2016).

CHEMICAL COMPOSITION OF BEANS IS ASSOCIATED WITH COFFEE QUALITY

The quality of the coffee drink is closely associated with the flavour and aroma, providing great satisfaction to the consumers who taste it. Commercial quality depends on the physical characteristics of the beans and sensorial characteristics of the beverage (Kitzberger et al., 2013; Sholz et al., 2013).

The definition of the quality of coffee as a beverage is quite broad, being dependent on the chemical composition of the beans, which is determined by genetic, environmental and cultural factors; harvesting, processing and storage methods; and roasting and preparation of the beverage. To obtain superior coffee, harvesting care and post-harvest management have become fundamental for commercialization and to increase the coffee grower’s profit (Coradi; Borém; Oliveira, 2008; Santinato et al., 2015). Therefore, it is essential that the harvested coffee be prepared and then subjected to drying to avoid fermentation processes, which will damage the quality of the beverage (Bozza et al., 2009; Compri et al., 2016).

To evaluate the quality of the beverage, the following parameters are considered to be efficient markers: soluble solids (Santos; Chalfoun; Pimenta, 2009; Ribeiro et al., 2014); total sugar content (Mendonça et al., 2007; Pérez-Hernández et al., 2012); electrical conductivity and potassium leaching (Malta; Chagas, 2009; Borém et al., 2013); total titratable acidity (Pimenta et al., 2008); and polyphenoloxidase activity (Mazzafera; Gonçalves; Shimizu, 2002; Angélico, 2011). Additional quality indicators include the official beverage classification (cup test), according to the CNNPA Resolution n° 12, 1978, and classification by type, which obeys the Official Table for Classification based on impurities and defects found in 300 g of coffee (Brasil, 2003).

Electrical conductivity and potassium leaching

The basic principle of the electrical-conductivity technique is to measure the amount of electrolyte released by the seed when soaked in water. This amount is directly proportional to the degree of disorganization of the plasma membrane, and the permeability of the membrane is highlighted as one of the fastest and most promising tests to evaluate the seed quality of several species (Kikuti et al., 2008; Dalvi et al., 2013; Douradinho et al., 2015).

The electrical-conductivity and potassium-leaching tests have been consistently used as efficient indicators of the integrity of cell membranes (Araújo et al., 2011) because these techniques present greater sensitivity in the detection of membrane degradation in coffee beans, which is caused by inappropriate handling in the pre- and post-harvest phases.

Soluble solids

In the food industry, the Brix scale is widely used to determine the soluble solid content present in a sucrose solution. The analysis is performed in a few seconds and requires a very small amount of sample (Zeferino et al., 2010).

In the case of coffee, greater amounts of soluble solids are desired, for industrial yield and to ensure a full-bodied beverage; interestingly, the use of cultivars that present a greater proportion of this fraction leads to high-quality drinks. This fraction is composed of water-soluble compounds, such as sugars, acids, vitamin C and some pectins, and may vary according to the type of processing, cultivar (different cultivars subjected to the same type of processing) and stage of maturation (Borém et al., 2008).

Total sugars

Sugars are associated with the quality of the coffee beverage and the sugar content depends mainly on the species and the place of cultivation of the coffee as well as the stage of maturation of the fruit. Sugars are associated with amino acids and proteins and are precursors of several volatile and non-volatile compounds (Messias et al., 2012).

During the roasting process, sucrose, which is the most prevalent sugar, is almost completely degraded, being used in the Maillard reaction and Strecker degradation, generating several volatile and non-volatile compounds. Among these products, acids and aldehydes are responsible for aroma, and caramelized sugars, are important for colour, viscosity and body attributes (Pérez-Hernández, et al., 2012; Ludwig et al., 2013).

Total titratable acidity

Acidity is a typical and, to some extent, desirable feature of coffee; however, at elevated levels, acidity can be considered a defect. Little is known about the acids found in the beverage and the influence of these acids on pH and perceived acidity (Vignoli; Bassoli, 2007).

The perceived acidity in coffee is an important attribute for sensorial analysis of the product, and the intensity of perceived acidity is influenced by several factors, such as climatic conditions during harvesting and drying, place of origin, type of processing and stage of maturation (Siqueira, Abreu, 2006). Perceived acidity can also assist in the evaluation of the quality of the coffee beverage (Scholz et al., 2013; Koskei; Patrick; Simon, 2015).

Polyphenoloxidase (PFO)

Polyphenoloxidase is a cupric enzyme of great importance in the evaluation of the quality-related attributes of various fruits and vegetables, and is the only enzyme known to catalyse the aerobic oxidation of phenolic compounds, which are some of the most influential determinants of quality, mainly flavour and aroma, of coffee and many plant products (Amorim; Silva, 1968; Mazzafera; Gonçalves; Shimizu, 2002).

Insect attacks, microbial infections, physiological alterations and mechanical damage before and after harvest induce rupturing of the cell membrane, resulting in greater contact between enzymes and the chemical compounds that act inside and outside the cells of the bean, leading to modification of the original composition of green coffee (Mazzafera; Gonçalves; Shimizu, 2002).

It is possible to determine the quality of the drink based on PFO activity. In addition, a table can be established with delimiting bands for each classification, such that the highest values would be associated with the best standards and the lowest values would be associated with the worst standards (Carvalho et al., 1994; Souza et al., 2013).

Roasting of coffee beans

The roasting of coffee is a process that is considered to be of prime importance for the quality of the final product since this process consists of heating the beans at high temperatures, promoting physical and chemical changes in the beans, such as changes in colour and aroma (Ruosi et al., 2012). Physical changes promote volumetric expansion and changes in bean texture and colour. As a result of the heat transfer, pyrolytic reactions occur, which cause the formation of several volatile and non-volatile compounds, mainly via the Maillard reaction, hydrolysis and condensation of compounds, and caramelization of sugars. These compounds are responsible for numerous sensory characteristics, such as fragrance, aroma, acidity, body, sweetness and residual flavour (Pérez-Hernández, 2013).

In a light roast, there is a predominance of a certain acidity due to the presence of chlorogenic acid, citric acid, malic acid and tartaric acid, the concentrations of which decrease with increasing degree of roasting, leading to a greater tactile perception of oiliness and viscosity in the mouth coffee beverage (olfactory perception caused by gases released from r and stronger aroma of the oasted and ground coffee after preparation of the infusion). In the medium roast, the body and aroma are more prominent, and in a dark roast, the burnt flavour is caused by the carbonization of some components of the coffee (Uejo Neto, 2008).

IMPORTANCE OF QUALITY PRESERVATION FOR THE MAINTENANCE OF BIOACTIVE COMPOUNDS IN COFFEE

In addition to macronutrients and essential micronutrients, a normal diet provides some chemical compounds, mostly present in fruits and vegetables, that have potent biological activity proven by several studies. These compounds are called bioactive compounds and may play several beneficial roles in human health, being extra-nutritional components and typically occurring in small amounts in foods (Monsalve et al., 2016).

Coffee has many bioactive compounds, and the consumption of coffee is associated with numerous beneficial health effects. The main bioactive compounds in coffee are chlorogenic acids, which are present at concentrations of 4 to 5.5% and are responsible for a series of volatile compounds that are important for the flavour of the beverage; caffeine, an odourless substance that has a very characteristic bitter taste, contributing an essential bitterness to the flavour and aroma to the coffee beverage; and trigonelline, which, during intense roasting, undergoes severe thermal degradation, generating a series of volatile compounds that are important for the taste and aroma of the beverage as well as an important vitamin for human metabolism, niacin (Kitzberge et al., 2013).

Epidemiological studies that address the ingestion of coffee present interesting results, suggesting that the daily intake of the beverage is capable of contributing to a reduction in the risk of developing non-communicable chronic diseases, such as cardiovascular diseases, metabolic disorders, cancers, neurodegenerative diseases and inflammatory diseases, in addition to improving alertness (Bidel et al., 2013; Ding et al., 2015; Lee et al., 2016; Bai et al., 2016).

MICROBIAL PARTICIPATION ON SENSORIAL PROPERTIES AND SAFETY OF COFFEE

As described in previous sections of this review, there are different kinds of coffee beverages, and consequently, there are differences in the chemical, physical and physicochemical compounds that confer different nuances in terms of body, aroma, acidity and astringency. The action of microorganisms is among the many factors that influence the final beverage (Esquivel; Jiménez, 2012; Pereira et al., 2014). The microbial metabolites produced in this period can diffuse into the grains and influence the final quality of the beverage. The microbial diversity in this process is high, and several species of bacteria, yeast, and filamentous fungi have been identified (Silva et al., 2008).

Pre-harvest

In extreme cases, when fruit ripening occurs under very favourable environmental conditions for microorganisms, undesirable fermentation can occur in the fruit while the fruit is still on the plant. An example of these extreme cases are plantations that are near water bodies, where the relative air moisture during coffee development is high. In this case, no amount of investment in post-harvest operations will restore the quality of the final product as the plant has already been compromised (Chalfoun; Fernandes, 2013).

Fermentation by the action of microorganisms such as bacteria, fungi and yeast degrades the membranes of the beans (Jones; Jones, 1984; Silva et al., 2013). However, if the process is stopped after the loss of the mucilaginous layer of the fruit, the endosperm is not compromised, and beverage quality is preserved (Silva, 2014). The continuity of the process involves breaking the walls and cell membranes, cell-layer degradation and alteration of the chemicals of the bean and beverage, resulting in an unpleasant taste and odour (Silva, 2014; Pereira et al., 2014).

Factors that cause pre-harvest damage, such as microbial infection of the fruit while still on the plant; insect attacks, mainly by the coffee fruit borer (*Hypothenemus hampei*), which injures the plant, facilitating infection by micro-organisms (Vega; Mercadier,1998); growth of soil microorganisms in the fallen fruit beneath the canopy (sweeping coffee); adverse weather conditions that cause injury to the fruit (frost, hailstorms); and excessively mature fruits that remain on the tree (in which senescence has already started), explain the difference in quality from one coffee region to another. In places where low-quality coffee is produced, weather conditions, such as high relative humidity during ripening, harvesting and processing of coffee and high temperatures, favour the further development of microorganisms (Velmourougane et al., 2010).

Coffee processing methods and differences in the microbiota

Coffee is processed in one of the following ways: dry, wet and natural pulped processing; the last processing method was developed in Brazil. The dry process is the predominant process used for Arabica coffees in Brazil, Ethiopia and Yemen as well as for practically all Canephora coffees worldwide. The wet process is the predominant method for Arabica coffees in Colombia, Costa Rica, Guatemala, Mexico, El Salvador, and Kenya and, as of recently, for a small percentage of Canephora coffees (Brando, 2004).

Microorganisms can be naturally present in all coffee post-harvest and influence the final quality of the beverage either by degradation of compounds present in the beans or by the excretion of metabolites. Microorganisms primarily affect the coffee when the sample contains a mixture of different stages of maturation and is processed by the dry method. Wet coffee processing, in which ripe fruit are subjected to rapid elimination of fermentation sources, if well conducted, may result in high quality coffee.

The dry process refers to methods in which the fruits are fully dried to yield coffees known as unwashed or natural coffees, which are immediately taken forward to drying after harvesting and hydraulic separation. Hydraulic separation, by which the fruit are separated in two fractions, is of fundamental importance; one fraction contains unripe and ripe fruits that are more dense, and the other contains fruit that are less dense due to different factors, such as biotic factors (bored fruit) and abiotic injuries (fruits that dried on the plant and are known as floaters).

Wet processing refers to various methods in which the beans are mechanically separated and the fresh fruit are peeled (pulping) before drying; wet processing may or may not include a fermentation step.

The method adopted depends on economic factors or environmental factors, such as high relative humidity during the harvesting and drying of the coffee. One should also consider the requirements and preferences of the intended markets for coffee production.

The vast majority of Brazilian coffee beans are still processed by the dry method since Brazil is one of the few countries in the world that has the appropriate weather to do so successfully. Due to Brazil's distinct dry and wet seasons, flowering and cherry maturation is homogeneous compared with countries such as Colombia, where flowering and consequent fruit ripening occurs all year long. These conditions allow Brazilians to harvest coffee via the strip-picking method and/or mechanically. Although under-ripe and over-ripe cherries are also harvested, careful processing can easily remove these coffee cherries (Chalfoun; Fernandes, 2013).

Coffee seeds have all the precursors needed to generate the typical flavour and aroma during the roasting operation, but the natural microbiota during the fermentation/drying step confers a special flavour to the coffee beverage. The microorganisms naturally present in the production environment use sugars in the pulp and mucilage and excrete organic acids and other metabolites that may affect the final sensory characteristics of the beverage. In addition, coffee fermentation and drying must be managed in order to control the growth of filamentous fungi that can alter the flavour and produce mycotoxins (Silva et al., 2014).

Microbiota present in natural processing

The production of natural coffee, traditionally known as the dry method, is the oldest and simplest coffee processing method and involves drying of the entire coffee fruit. This method is largely used in tropical regions where the dry season coincides with the harvest period (Borém; Isquierdo; Taveira, 2014). In some cases, depending on factors such as plantation size and occurrence of rain during the harvest period, the cherries are machine dried after being dried in the sun for a few days. However, with respect to coffee quality, the presence of any fruit tissue, such as exocarp, skin or pulp containing the mucilaginous layer, may predispose the beans to infection by microorganisms, including toxigenic microbes.

The dry process has more microbial species than the other processes (pulped or dehulled) because the whole fruit is processed, and it is known that in dry method, the detrimental microorganisms, present superficially on the skin, have more opportunity to penetrate the pulp and reach the beans. On the other hand, in the wet process, the skin and pulp are fully or partially removed, and consequently, the epiphytic microorganisms are mechanically removed.

The presence of organic acids from fermentation (acetic, lactic, butyric, and propionic acids) confirms microbial activity during the fermentation process for natural coffees. In the beginning of the process, bacterial species predominate over yeast and filamentous fungi, but the microbial composition changes when water content and physicochemical conditions change in the medium during the final phase of fermentation.

Silva et al. (2008), studying the succession of fungal and bacterial communities during the fermentation of natural coffee (*Coffea arabica* L.), found that *D. hansenii* and *Pichia* were the most abundant among the yeasts but were present with smaller populations than those of fungi and bacteria. However, the yeasts identified in this study have been reported to inhibit the mycelial growth of filamentous fungi and thus may potentially be used for the biocontrol of filamentous fungi.

Other tests have been developed to evaluate the improvement of coffee beverage quality by using selected yeast strains during fermentation in the dry process, with promising results observed using the selected yeasts as starters in the coffee fermentation process (Silva, 2013; Evangelista et al., 2014).

Microbiota present in wet processing

According to Silva, 2014, the microbial diversity of coffee beans in wet processing is smaller than that in dry processing because the fermentation time is shorter (up to 48 h) and there is a more rapid decline in pH from 6 to 4.3. This microbiota comprises a few bacterial and yeast species. There is no report of filamentous fungi involved in wet fermentation. The mucilage adhered to the substrate beans is used by bacteria and yeast during fermentation (Silva, 2008; Batista, 2009).

Regarding the role of yeast in the fermentation of depulped beans, some researchers do not believe that yeast action leads to mucilage degradation. However, pectinolytic yeasts have been isolated during the wet processing of both Robusta and Arabica coffees. Pereira et al., 2014, used a selected yeast starter culture, *Pichia fermentans* YC5.2, originally isolated from wet processing and selected based on specific characteristics suitable for fermentation, viz., coffee fermentation-associated stress tolerance and flavour-active ester compound production.

Microbiota present in semi-washed processing

The pulped natural method consists of pulping the coffee but omitting the fermentation step to remove the silver skin. The microbiota present in natural dehulled coffee processing is still not well known. The great diversity of prokaryotes and eukaryotes was detected using polymerase chain reaction/denaturing gradient gel electrophoresis (PCR-DGGE) in addition to conventional techniques. PCR-DGGE allowed the detection of species not isolated by conventional techniques, such as *Enterobacter cowanii, Lactobacillus plantarum, Pantoea agglomerans, Bacillus macerans*, and an endophytic bacterium that could not be cultivated as well as the yeasts *S. cerevisiae and H. uvarum*. The presence of filamentous fungi was detected at population sizes below 10^3 CFU $^{g-1}$ and only in recently harvested fruits, washed fruits, and fractions of skin and pulp.

The filamentous fungi species detected were *Aspergillus sp., A. chevalieri*, *A. foetidus, A. niger, A. ochraceus, A. tubingensis, A. versicolour, Cladosporium sp., C. cladosporioides, C. macrocarpum, Cylindrocarpon sp., Eurotium chevalieri, Fusariella sp., Fusarium sp., F. chlamydosporum, F. lateritium, F. nivale, F. solani, F. sporotrichioides, Geotrichum sp., Mucor hiemalis, Penicillium sp., P. brevicompactum, P. commune, P. decumbens, P. fellutanum, P. implicatum, P. roqueforti, Phoma sp.*, and *Ulocladium sp.* (Silva, 2014; Chalfoun; Fernandes, 2013).

Microorganisms of that cause coffee spoilage and microorganisms that produce mycotoxins

Stringent food safety standards may be set by specific importers, country-specific bodies and global standard organizations. The concept that one of the most important components of food quality is safety is also becoming popular among consumers. Coffee contains a set of molecules that can affect health; some of these molecules are naturally present in coffee beans, while others are derived from biochemical reactions that occur during roasting. In addition, molecules such as ochratoxin A and pesticide residues are external compounds that are independent of coffee chemical composition.

According to Scussel (2002) and Taniwaki (2003), fungi, including toxigenic fungi, can infect coffee plants and grow in the beans in the field during harvesting, drying and storage. The factors that favour the growth of these fungi and the production of mycotoxins are classified into three categories: physical, chemical and biological. These factors are related to the bean itself and to the environment that surrounds the bean. The most important of these factors are the following: bean moisture content, relative humidity, air temperature, strain of contaminant fungus and microbial competition. Storage period, impurities (plant debris, dust, bark and bits of bean), light, insects and mites, bean conditions (beans with mechanical and/or visible damage), microclimate (oxygen), fungicides, substrate composition, resistant varieties and degree of contamination can promote the proliferation of fungi and production of mycotoxins (Scussel, 2002; Pasin, 2009).

Three species or groups of species, all of the genus Aspergillus, are of significance: The *A. niger* complex is undoubtedly the most common, particularly in *Coffea canephora* (Robusta), but OTA production is rare and usually feeble. A study revealed only one OTA producer among seventy isolates tested; *A. ochraceus* and related fungi are well distributed in coffee production systems and are common producers of OTA (approximately 80% of the isolates readily produce OTA), constituting most important group of species for mycotoxin production; *A. carbonarius* is usually rare, but there is some evidence that this species is relatively common in certain places. Many isolates seem to be capable of producing OTA in significant quantities, although in a limited range of environmental conditions. None of the OTA-producing species of the genus *Penicillium (P. verrucosum* and *P. nordicum*) have been isolated from coffee. *P. brevicompactum* is common in coffee and is in the same group as the two OTA-producing species but is not a producer of OTA (Batista, 2009).

Ochratoxins are a group of potent renal mycotoxins that widely contaminate agricultural products such as corn, wheat, oats and dried beans. There are four ochratoxin homologues - A, B, C and D. Ochratoxin A (OTA) is the most prevalent and, together with ochratoxin C, the most toxic. Initial symptoms of ochratoxicosis observed in all species include anorexia, polydipsia, polyuria and dehydration, and ochratoxicosis is associated with renal damage.

Upon absorption, ochratoxins enter the circulatory system, bind tightly to serum proteins and accumulate in the kidneys, where they disrupt protein synthesis and other pathways in proximal tubular cells. This phenomenon results in the degeneration of proximal tubules and interstitial fibroses (Krogh, 1992). OTA is also known to bind to DNA molecules and induce renal tumours in animal models, although the carcinogenic mechanism of OTA remains controversial (Faucet et al., 2004; Mally et al., 2004; Reddy; Bhoola, 2010; Bui-Klimke; Wu, 2015).

The European Union, in order to control the presence of OTA in coffee beans and their derivatives, has recommended maximum tolerance levels of 5 µg/kg for ochratoxin A in roasted and ground coffee and 10 µg/kg in instant coffee for sale (applicable since April 2005). Brazilian legislation establishes a maximum tolerable limit of 10 µg/kg of mycotoxin for ground, whole bean and instant coffee, as approved by resolution RDC Nº 7 of the National Health Inspection Agency (Agência Nacional de Vigilância Sanitária or ANVISA) on February 18, 2011 (Chalfoun; Parizzi, 2014).

Thus, Good Agricultural Practices (GAP) and Good Manufacturing Practices (GMP) procedures are needed to control possible sources of contamination and to ensure that the product meets the sensorial quality and safety specifications. These procedures include aspects ranging from the conditions of production to the processing and storage facilities as well as personal hygiene. These aspects are fundamental prerequisites and are becoming the hygienic and sanitary basis for the implementation of the Hazard and Critical Control Point Analysis System (HACCP) and meet the need for quality assurance and security, which is increasingly required for food products.

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