

# JOEL DOUGLAS SHULER

# EFFECT OF THE PRESENCE OF THE PERICARP ON THE CHEMICAL COMPOSITION AND SENSORIAL ATTRIBUTES OF ARABICA COFFEE

LAVRAS – MG 2017

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Dissertation presented to the Universidade Federal de Lavras as part of the requirements of the Post-Graduate Program in Agriculture Engineering, Processing of Agriculture of Products concentration, for obtaining the title of Master.

Prof. Dr. Flávio Meira Borém

Graduate Advisor

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#### ABSTRACT

The purpose of this study was to gain further insight into how the natural coffee flavor profile develops during processing. This was done by analyzing how the presence of the pericarp, removed at different times throughout the coffee drying, affected the chemical composition of green coffee beans and the resulting flavor in the coffee beverage. Coffee fruit was selectively harvested from a highaltitude coffee field outside of Cristina, Minas Gerais, Brazil, picking only ripe fruit. This fruit was subjected to hydraulic separation of floaters and then further hand selection to ensure the lot contained only ripe, undamaged coffee fruit. Part of the lot was separated and processed as a wet process coffee. It was pulped, removing the epicarp and part of the mesocarp, and manually washed with fresh water to remove any remaining mesocarp (mucilage). The remaining fresh fruit was divided into 5 treatments, a treatment being defined by the wet basis moisture content level at which its pericarp would be removed  $(32 \pm 2 \degree C, 28 \pm 2 \degree C, 22 \pm 2 \degree C, 18 \pm 2 \degree C$  and  $11 \pm 2 \degree C$ ). All samples were dried at a temperature of  $37 \pm 2$  °C with constant rotation during the drying, first every half hour, then every hour once 32% moisture content was reached, and then every two hours once 18% moisture content was reached. After one month of storage, the coffee was hulled and then subjected to chemical and sensory analysis. The chemical analysis comprised bioactive compounds (trigonelline, caffeine, and total chlorogenic acids), low molecular weight carbohydrates, fatty acids, and organic acids. The results yielded statistically significant differences in levels of trigonelline, quinic acid, and malic acid. Perhaps as important, the results showed no difference in either the levels of fatty acids or low molecular weight carbohydrates. The overall scores for the sensory analysis were not statistically different; however, no taster (cupper) identified the wet process coffee as having a "natural flavor profile."

Keywords: Coffee Processing. Coffea arabica L. Pericarp Effect on Coffee Bean.

#### RESUMO

A proposta, neste trabalho, foi obter mais informações sobre como o perfil de sabor do café natural se desenvolve durante o processamento. Para isso, foi analisado como a presenca do pericarpo, removido em diferentes tempos de secagem do café, afetou a composição química do grão de café verde e os resultados do sabor na bebida do café. Frutos de café foram coletados seletivamente de uma lavoura cafeeira situada em área de altitude elevada, no município de Cristina, estado de Minas Gerais, Brasil. Somente frutos maduros foram coletados. Os frutos foram submetidos à separação hidráulica de boias e, em seguida, selecionados manualmente para garantir que o lote contivesse apenas frutos maduros e não danificados. Parte deste lote foi separada e processada por via úmida do café. Frutos foram despolpados, removendo-se o epicarpo e parte do mesocarpo, e lavados manualmente em água fresca para remover algum mesocarpo remanescente (mucilagem). Em seguida, estes frutos foram divididos em cinco tratamentos definidos pelo teor de água do grão na hora da remoção do pericarpo (32±2 °C, 28±2 °C, 22±2 °C, 18±2 °C e 11±2 °C). Todas as amostras foram secas à temperatura de 37±2 °C, sob rotação constante durante a secagem, primeiro a cada meia hora, depois a cada uma hora, até obter 32% de umidade, e a cada 2 horas até obter 18% umidade. Após um mês de armazenamento, o café foi descascado e submetido às análises químicas e sensoriais. As análises químicas compreenderam compostos bioativos (trigolina, cafeína e ácido clorogênico total), carboidratos de baixo peso molecular, ácidos graxos e ácidos orgânicos. Os resultados apontaram para diferença estatística significativa nos teores de trigolina, ácido quínico e ácido málico. Com a mesma importância, os resultados não demonstraram diferença estatística significativa para os teores de ácidos graxos e carboidratos de baixo peso molecular. Os resultados globais das análises sensoriais não apresentaram diferenca estatística significativa, entretanto, nenhum provador (cupper)identificou o café processado pela via úmida como tendo um perfil de sabor de um café natural.

Palavras-chave: Processamento do café. Coffea arabica L. Efeito do pericarpo no grão de café.

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# **1 INTRODUCTION**

The demand for coffee is increasing worldwide. Within this demand, the segment of high quality coffees, loosely defined as "specialty coffees," is growing at an even faster rate. Many consumers are becoming more educated and consequently more demanding about the quality of their coffee purchases.

One of the key steps in coffee's journey from seed to cup is that of processing: the drying of the coffee in preparation for hulling. Historically, this has been done in two basic ways. The "dry process" entails drying the entire fruit intact, with the resulting coffee termed "natural." The "wet process" entails removing the outer layers of the fruit and drying just the parchment coffee, with the resulting coffee termed "washed" (BORÉM; ISQUIERDO; TAVEIRA, 2014; BRANDO, 2009).

It is well established that these two methods yield distinct flavor profiles (SELMAR; KLEINWÄCHTER; BYTOF, 2015; TEIXIERA et al., 2005). Yet exactly why these flavor profiles are different has not been fully explained.

Much of the historical and industry literature attributes these differences to different raw material, as historically only fully ripe coffee fruit is used with the wet process, since the pulpers used in this process will not pulp unripe or overripe fruit. With the dry process, on the other hand, various maturation levels can be and often are used (CLARKE, 1985; PUERTA-QUINTERO, 1996; VINCENT, 1987). However, while this may provide some insight into how these coffees are different in a general sense at the market level, it does not explain why the two different processing methods using the same raw material will yield coffees with two distinct profiles.

Perhaps the most common explanation given is that the sweeter, fuller-bodied flavor profile of naturals is due to the presence of the sugar-laden mucilage during drying. Yet proof has never been given that these sugars, in fact, make their way from the fruit into the bean, let alone the pathway by which they might do so.

More recently several theories have emerged that partially explain the different flavor profile rendered by the wet process. One promising line of research addresses the fact that removing the pericarp, as is done with the wet process, also removes inhibitors to germination, thus unlocking different metabolic processes associated with germination (SELMAR; KLEINWÄCHTER; BYTOF, 2015). However, no research exists in the literature analyzing the commonly held belief that the presence of the pericarp during drying impacts the chemical composition, and thus the flavor profile, of the coffee bean.

Given both the acceptance of high quality dry process coffees within the specialty coffee industry, as well as the growth of this industry, it is important that growers better understand how the natural coffee flavor profile develops, and ultimately how certain flavor profiles can be controlled and replicated. The aim of this study was to better understand the development of the natural flavor profile during post-harvest by looking at how the presence of the outer layer of the coffee fruit during the drying process altered the chemical composition of the coffee bean and the flavor of the resulting coffee beverage.

#### **2 LITERATURE REVIEW**

#### 2.1 The specialty coffee market and changing views of naturals

Over the last decade there have been many calls for coffee growers to improve their coffee quality so that they can take advantage of the new opportunities and higher margins that the growing specialty coffee industry provides. Yet the term "specialty coffee" still means different things to different people. In a technical sense, a specialty coffee is defined by the Specialty Coffee Association of America, SCAA (2015), as a coffee that scores at or above eighty (80) points using the SCAA Cupping Protocol, and has no primary defects and up to five (5) secondary defects using the SCAA green grading protocol. It has been estimated that only 5% of the coffee produced worldwide meets this standard (THE COFFEE..., 2011).

The term specialty coffee was originally used in a general sense to describe a range of coffee products sold by dedicated coffee shops to differentiate those coffees from the *other* coffee generally available through supermarkets and other retail outlets. Through its rapid growth and expansion, specialty coffee continued to define itself as other, a vague term that allowed specialty coffee to be an umbrella term for anything that was different. This other eventually developed into a plethora of *others*, and the term has become so broad that there is now no universally accepted definition as to exactly what it defines (STEIMAN, 2013).

Given the lack of an accepted definition of specialty coffee, it is impossible to quantify the exact market share and growth of the specialty coffee segment within the larger coffee market. However, despite the lack of precise quantification, the growth in the number of coffee shops, a barometer for the specialty coffee industry, is increasing. Another indicator that consumers are becoming more knowledgeable about coffee is the growing number of Q Graders, or certified coffee tasters, worldwide. In 2005, there were 29 certified Q Graders; by 2015, that number had grown to 4474 (COFFEE QUALITY INSTITUTE, 2005, 2015).

This growth in specialty coffee has been accompanied by a changing perspective on naturals. On June 14, 2016, Hacienda La Esmeralda, considered by many to produce some of the finest coffees in the world, sold its top lot of coffee for a staggering US \$135.00 per pound, over 80 times the commodity price of US \$1.6486 per pound paid the same day for "Other Mild Arabicas," the ICO classification for wet process coffees from Panama (ICO INDICATOR PRICE, 2016). However, the comparison is not exactly accurate, as the processing method used for that coffee was the dry process. While the ICO does not have a category for Panamanian naturals, the price paid that day for "Brazilian Naturals," an ICO

category including dry process coffees from Brazil, Ethiopia, and Paraguay, was US \$1.3741 per pound, or 16.6% lower than the price paid for "Other Mild Arabicas" (INDICATOR PRICE, 2016). While the commodity price for dry process auction was 16.6% lower, the average price paid for dry process coffee at the Esmeralda Special auction was \$109.72 per pound, 95.5% higher than the average price paid for wet process coffees. In fact, the highest price paid for a wet process coffee in the auction was US \$71.00, \$20 less than the lowest successful bid for a natural. One week earlier, the highest bid coffee from Finca Santa Felisa, a farm in Acatenango, Guatemala, known for its quality, was for a natural process coffee as well.

Such price discrepancy, with naturals fetching some of the highest prices in the world while receiving the lowest price of the three defined ICO coffee groups, can largely be attributed to the quality variation within the natural process. While naturals can be complex and clean coffees when the raw material is of high quality and the post-harvest is properly conducted, they are often inferior coffees, with raw material of inferior quality (e.g., various maturations) and/or less-than-optimal post-harvest treatments (e.g., drying at high temperatures). These conditions result in coffees that are prone to a larger number of physical defects, as well as off flavors such as ferment, phenol, and mold in the cup.

# 2.2 Coffee fruit anatomy and chemical composition

Before looking at processing and the effects of processing on the chemical and sensorial characteristics of coffee, it is first necessary to understand the anatomy and chemical composition of the coffee fruit and seed. The anatomical components of coffee can be divided into two main categories, the pericarp and the seed.

### 2.2.1 Pericarp

The pericarp comprises the outer layers of the coffee fruit: exocarp, mesocarp, and endocarp.

# a. Exocarp

The exocarp, also called the epicarp, commonly called the skin or peel, is the outermost tissue of the coffee fruit. It is composed of parenchyma cells (cells with thin cell walls). The exocarp is green for most of the fruit's development. Toward the end of

maturation, chlorophyll pigments disappear, and after a brief transient yellow phase, the exocarp cells accumulate anthocyanin, bringing on a red coloration. In the case of yellow fruit, leucoanthocyanin replaces anthocyanin, allowing exposure of the yellow pigment luteolin.

# b. Mesocarp

The mesocarp, also called the mucilage, is the fleshy part of the fruit between the parchment and the skin. In general, it makes up 22% to 31% of the mass of the dry fruit.

The mesocarp is hard in unripe coffee fruit. As the coffee matures, pectinolytic enzymes break down pectin chains, resulting in a hydrogel that is insoluble, colloidal (contains small particles that are evenly distributed throughout), hyaline (glassy), and rich in sugars and pectins. This difference is fundamental in the pulping process, as it allows for the separation of unripe and ripe fruit.

The mesocarp is commonly called "pulp." In some literature, it is referred to as the "true pulp" and in other literature it is divided into an inner mesocarp, called mucilage, and an outer mesocarp, which is called the pulp. However, in practical terms, the pulp is the exocarp, the part of the mesocarp that is removed during the pulping process.

Arabica coffee var. Typica <sup>1</sup>		Canephora coffee var. Robusta <sup>2</sup>		
Component	Content (w.b.)	Component	Content (w.b.)	
Dry Material	93.07%	Non-nitrogen extract	57.9%	
Carbohydrates	74.10%	Crude fiber	27.7%	
Non-nitrogen extract	59.10%	Reducing sugars	12.4%	
Crude fiber	15.10%	Crude protein	9.2%	
Crude protein	8.25%	Pectic substances	6.5%	
Ash	8.12%	Tannins	4.5%	
Moisture Content	6.93%	Ash	3.3%	
Tannins	3.70%	Non-reducing sugars	2.0%	
Potassium	3.17%	Lipids	2.0%	
Ether extract	2.50%	Chlorogenic Acids**	1.61%	
Nitrogen	1.32%	Caffeine**	0.54%	
Caffeine	0.75%			
Calcium	0.32%			
Phosphorus	0.05%			
Chlorogenic Acids**	1.1%			

Table 1. Pulp compositions of Arabica and Canephora coffee.

Source: (BORÉM; GARCIA SALVA; AMARAL DA SILVA, 2014).

# c. Endocarp

The endocarp, more commonly called the parchment, is made up of sclerenchyma cells (woody cells that serve as support structures in plants). The endocarp completely envelops the seed. It is mostly cellulosic material: 50% cellulose, 20% hemicellulose, and 20% lignin.

## 2.2.2 Seed

The coffee seed, or bean, comprises the silverskin, endosperm, and embryo. It should be noted that coffee seeds are considered to have "intermediate" storage behavior, meaning that their storage potential is between that of orthodox seeds (seeds that can tolerate significant drying, to at least 5% moisture content, without damage) and recalcitrant seeds (seeds that are unable to tolerate more than a limited amount of desiccation) (ELLIS; HONG; ROBERTS, 1991).

# a. Perisperm

The perisperm, also called the silverskin or spermoderm, is the outermost layer of the seed and is composed of sclerenchyma cells. Little is known about its molecular physiology or the roles it plays in seed development (BORÉM; GARCIA SALVA; AMARAL DA SILVA, 2014; EIRA et al., 2006).

The perisperm originates from the nucellus, and from the initial stages of seed develop until around 90 to 100 days after flowering (DAF), the spermoderm undergoes intense cell division and expansion, and it is the spermoderm, rather than the endosperm, that initially fills most of the locule space (DE CASTRO; MARRACCINI, 2006; GEROMEL et al., 2006). From 90 to 150 DAF, two layers of the perisperm can be distinguished: an inner layer with large cells and an outer layer formed by smaller cells. By 150 to 200 DAF, the perisperm has been reduced to a thin green testa (seed coat). As the fruit matures, the silverskin transforms into a thin pellicle (membrane or film) that may become partially detached upon drying (DE CASTRO; MARRACCINI, 2006). This difference is used in the classification of coffee to determine the presence of "unripes."

During coffee fruit development, the perisperm is tightly connected to the endocarp by the funicle. At the boundary between the perisperm and funicle, symplastic tissue (tissue that readily permits diffusion of water and low molecular weight solutes) is found in continuity with xylem and phloem vascular tissues that enter directly into contact with the perisperm. No vascular connection was observed between the pericarp and the perisperm. Since the endocarp is a thick cellulosic tissue even early in fruit development, any vascular connection that might exist between the pericarp and perisperm would necessarily be through the peduncle (GEROMEL et al., 2006).

## b. Endosperm

The endosperm is the principle reserve tissue for initial plant growth after germination. It is a living tissue that is hard due to high content of polysaccharides that are deposited in the cell walls.

The external part of the endosperm is composed of small polygon-shaped cells that are rich in oils and is sometimes called the "hard endosperm." The internal part of the endosperm, sometimes referred to as the "soft endosperm," is composed of larger rectangular cells with slightly thinner cell walls.

The polysaccharides in the cell wall account for 50% of the dry weight of coffee (15% cellulose, 25% to 30% arabinogalactan proteins, 50% mannan and galactomannan, and 5% pectin. Sucrose is the most abundant of the low molecular weight sugars (monosaccharides and disaccharides) and is found in concentrations up to 400 times the combined concentrations of the other sugars. Lipids make up between 12% to 18% of Arabica coffee beans, and 75.2% of these lipids are triglycerides. Proteins make up 9.2% of the dry weight of Arabica coffee beans (BORÉM; GARCIA SALVA; AMARAL DA SILVA, 2014).

# c. Embryo

The embryo is small (3–4 mm long) and is composed of an axis attached to two cotyledons. It is located close to the convex surface of the seed. It contains few storage reserves and is therefore dependent upon the endosperm for nutrients during its initial growth.

### 2.3 Analysis of certain chemical compounds found in green coffee

The presence of certain chemical compounds within the green coffee beans is important to the flavor profile as well as potential health benefits of the roasted coffee.

#### 2.3.1 Bioactive compounds

Bioactive compounds, while not essential nutrients, nonetheless affect living organisms. Recently their study has been emphasized, given their potential health benefits. In coffee, three bioactive compounds frequently studied are trigonelline, caffeine, and chlorogenic acids.

#### a. Trigonelline

Trigonelline is a pyridine alkaloid that has been considerably researched, as its thermal degradation during roasting leads to significant sensory and nutritional components. Most studies report its levels in Arabica around 0.6% to 1.77%; however, an analysis by Mazzafera of 14 different Arabica cultivars found a range between 1.97% and 2.48% (KY et al., 2001; MACRAE, 1985; MAZZAFERA, 1991).

Though it is normally completely extracted from the roasted coffee at standard brewing temperatures, trigonelline has little direct impact on the flavor of the coffee beverage. This is due partly to its significant degradation during roasting. The loss of trigonelline varies per roast conditions, with losses between 50% and 90% reported in the literature (FARAH et al., 2006; MACRAE, 1985). The bitter taste of trigonelline is weak, estimated to be a quarter that of caffeine, which itself only contributes to 10% of the bitterness tasted in coffee (MACRAE, 1985).

It is largely the thermal degradation (demethylation) of trigonelline during roasting that makes it an important compound in green coffee, both from a nutritional and a sensory standpoint. Nutritionally, the thermal degradation of trigonelline results in nicotinic acid, or niacin, a B<sub>3</sub> vitamin that is an essential nutrient to humans. Though this represents only a small part of trigonelline loss during roasting, the nutritional impact can be significant. For example, a medium-roasted 200 ml cup of coffee provides 40% of the Daily Recommended Intake (DRI) of Niacin (FARAH, 2009). Like caffeine, trigonelline may be toxic; however, its lethal dose is far inferior to that of caffeine and is well below the amount ingested in coffee consumption.

In studies of the thermal degradation of coffee during roasting, it was determined that among the various resulting compounds, the non-volatile fraction comprised Nicotinic acid, *N*-methlynicotinamide, and methylnicotinate, while 29 volatiles were found, nine of which were identified in coffee aroma (VIANI; HORMAN, 1974).

One study showed a positive association between trigonelline levels and good cup quality when examining correlations between various qualities of Brazilian coffee (FARAH et al., 2006). However, since the lower cup quality levels in this study were from defective coffee, presenting various intensities of the Rio defect, it should not be inferred that a similar correlation could be used to separate qualities of higher quality, non-defective coffee (i.e., to differentiate the "good" from the "great").

Several studies, using selectively harvested coffee with no defects, demonstrated a positive association between trigonelline and higher altitudes (FIGUEIREDO et al., 2013; RIBEIRO, 2013; RIBEIRO et al., 2016).

## b. Caffeine

Caffeine (1,3,7-trimethylxanthine), perhaps coffee's best-known chemical compound, is a methlyxanthine alkaloid found in the seeds, leaves, and fruits of numerous plants. It is likely that caffeine plays a role in coffee plant defense, as studies have described its roles as a fungicide (ARORA; OHLAN, 1997; RAUT et al., 2013), phytotoxin (HOLLINGSWORTH; ARMSTRONG; CAMPBELL, 2003), and chemosterilant to certain insects (RIZVI; PANDEY; MATHUR, 1980).

Average values for commonly commercialized Arabica coffee have largely been reported in the literature, with ranges from 0.96% to 1.90% in most Arabicas (ASHIHARA; SANO; CROZIER, 2008; DESSALEGN et al., 2008; KY et al., 2001; MACRAE, 1985), though some varieties of Arabica have significantly lower quantities (CARVALHO et al., 2008; MENDES et al., 2008; SILVAROLLA; MAZZAFERA; FAZUOLI, 2004).

Caffeine presents significant stability, both in terms of lack of mobility within the coffee plant (MAZZAFERA; GONCALVES, 1998) and in its consistent levels throughout both fruit and seed development (DE CASTRO; MARRACCINI, 2006), processing (DUARTE; PEREIRA; FARAH, 2010; JOËT et al., 2010; LELOUP et al., 2005), and subsequent roasting (CASAL; BEATRIZ OLIVEIRA; FERREIRA, 2000; FRANCA et al., 2005; MACRAE, 1985). While roasting temperatures generally exceed caffeine's sublimation point (178 °C) caffeine losses in roasting are insignificant, probably due to pressure build-up within the bean and a poor rate of vapor diffusion through the bean's outer layers (MACRAE, 1985).

Caffeine has a bitter taste; however, it has been reported that caffeine only contributes 10% to the overall bitterness of the coffee beverage (FLAMENT, 2002).

In studies comparing defective and non-defective coffee, some studies showed an association of higher levels of caffeine with higher cup quality (FARAH et al., 2006; FRANCA; MENDONÇA; OLIVEIRA, 2005). However, another study by one of the same authors shows lower levels of caffeine in non-defective beans compared to black, immature, and sour defects (FRANCA et al., 2005).

A study of 42 Arabica genotypes presented a significant negative association between caffeine levels and cup quality, specifically cup quality attributes of acidity, body, flavor, and overall (DESSALEGN et al., 2008).

# c. Chlorogenic Acids

Chlorogenic acids are the main phenolic compounds in coffee, playing an important role in coffee flavor development and as an antioxidant contributing to coffee's potential health benefits. The chlorogenic acids, normally caffeic, ferulic, and *p*-coumaric, are esters of quinic acid and cinnamic acid, and constitute a quantitatively important class of compounds in coffee (BORÉM; GARCIA SALVA; AMARAL DA SILVA, 2014).

Chlorogenic acids are divided into classes based on the nature and number of cinnamic substituents and their esterification position in the cyclohexane ring of quinic acid. The following major classes of chlorogenic acids are commonly found in green coffee beans: caffeoylquinic acids (CQA), dicaffeoylquinic acids (diCQA), feruloylquinic acids (FQA), *p*-coumaroylquinic acids (CoQA), and caffeoylferuloylquinic acids (CFQA) (CLIFFORD, 1985; PERRONE et al., 2008). Several additional minor classes, including three isomeric dimethoxycinnamoylquinic acids, three caffeoyl-dimethoxycinnamoylquinic acids, three diferuloylquinic acids, and three feruloyl-dimethoxycinnamoylquinic acids, were recently detected and characterized in green coffee beans; however, they make up less than 1% of the total CGA content (CLIFFORD et al., 2006).

A study on the content of bioactive compounds in wild Arabica coffee found the percentage of dry matter for each of the major classes to be (parentheses show percentage of total CGA): CQA 3.26% (80%); diCQA 0.60% (15%); and FQA 0.19% (5%). Of the various isomers, 5-CQA was found in the greatest quantities, making up on average 75.3% of the total CGA content (KY et al., 2001). In a study on the correlation of cup quality and chemical attributes in Brazilian coffee, CQA represented 83% of the total CGA (FARAH et al., 2006). Similar results were achieved in a separate study with CQA, diCQA, and FQA representing 84%, 11%, and 4% of total CGA, respectively (PERRONE et al., 2008).

CGA degrades significantly during roasting, with studies showing losses ranging from 27% in lighter roasts to over 96.5% for darker roasts (PERRONE et al., 2008; TRUGO; MACRAE, 1984). Thermal degradation of chlorogenic acids results in free phenolic acids, and consequently volatile phenols that contribute to the aroma and taste of roasted coffee. Phenols have been associated with the aromas of smoky, burnt, spicy, clove-like, and bitter, as well an astringent taste (DART; NURSTEN, 1985). Quinic acid is formed from CQA when roasting; however, its resulting quantities cannot account for all of the extractable CGA that is destroyed during roasting (CLIFFORD, 1985).

CGA content is associated with fruit maturity, with total CGA increasing during fruit development and peaking four weeks before full maturity. Frequently, immature beans have a higher diCQA content than fully mature beans (CLIFFORD, 1985).

Studies have shown different associations of CQA isomers with coffee quality and altitude. A study examining correlations between coffee quality and chemical composition found that 3-4-diCQA levels in green and roasted coffee were associated with high quality, while higher levels of caffeoylquinic acids, mainly 5-CQA, and FQA, were associated with poor cup quality (FARAH et al., 2006). A study examining climatic effects on chemical composition found that 3-CQA and 4-CQA as well as di3.4-CQA and di.4.5-CQA were positively correlated with temperature, while the opposite trend occurred for both 5-CQA and di-3.5 (JOËT et al., 2010).

#### 2.3.2 Organic acids

As their name implies, organic acids are acids that contain carbon atoms. The most common group of organic acids are carboxylic acids, which contain a COOH carboxyl group. They are important components in food both for their sour flavor, and for their role in food preservation due to their antibacterial effects.

Organic acids have been found in Arabica coffee in the following concentrations (w.b.): acetic acid traces 0.058%; citric 0.50%–1.58%; malic 0.26%–0.67%; quinic 0.33%–0.70%; succinic 0%–0.74%; and phosphoric 0.11%–1.15% (ALCÁZAR et al., 2003; JHAM et al., 2002).

Roasting significantly impacts organic acids, leading to increases in the levels of some and decreases in others. A major fraction of the acidity in roasted coffee can be attributed to the formation of four aliphatic acids during roasting: formic, acetic, glycolic, and lactic. Sucrose is the primary precursor to these, and increased levels of sucrose, glucose, and fructose in the green beans have been directly correlated to increases in these four acids (GINZ et al., 2000). Another acid that increases during roasting, though to a lesser degree, is quinic acid, which increases through CGA degradation (BALZER, 2001; CLIFFORD, 1985). Other acids continually decrease throughout roasting, including citric and malic acid (BALZER, 2001).

Different organic acids add different flavor elements to coffee. For example, citric acid is usually identified with various citrus fruits such as orange or lemon, lactic acid with butter, and malic acid with green apples (LINGLE, 2011).

# 2.3.3 Fatty acids

The principle compounds of lipids are fatty acids, which are made up of a long aliphatic chain and a carboxylic acid group. They are usually derived from triglycerides or phospholipids. Fatty acids are found in coffee in quantities ranging from 10% to 17%. Most of the lipid fraction is found in the cytoplasm of the reserve cells of the endosperm, though a small quantity (0.2% to 0.3%) is found in a waxy layer that envelops the bean (BORÉM; GARCIA SALVA; AMARAL DA SILVA, 2014; FOLSTAR, 1985).

The fatty acids found in the highest concentrations in coffee are myristic (C14:0), palmitic (C16:0), stearic (C18:0), oleic (C18:1), linoleic (C18:2), linolenic (C18:3), arachidonic (C20:0), gadoleic (C20:1), behenic (C22:0), and lignoceric (C24:0) (SPEER; KOHLLING-SPEER, 2006).

The lipid fraction of coffee is stable and undergoes only minor changes during roasting (BELITZ; GROSCH; SCHIEBERLE, 2009). However, the thermal oxidation of the lipid fraction produces further compounds, in particular aldehydes, that react with intermediaries of the Maillard reaction, generating additional aroma compounds in the coffee (FLAMENT, 2002).

Lipids play an important part in the flavor of the coffee beverage. Even when their concentration is low, their high reactivity may have a large impact on organoleptic qualities (BELITZ; GROSCH; SCHIEBERLE, 2009). The oxidation of lipids has also been indicated as a major contributor to the staling taste (DUSSERT et al., 2006; SPEER; KOHLLING-SPEER, 2006).

Several studies have addressed the association between lipid content, genotype, and environmental conditions (BERTRAND et al., 2008; VILLARREAL et al., 2009); however,

no consensus exists as to the association of total fatty acids or fatty acid profiles and coffee quality.

#### 2.3.4 Low molecular weight carbohydrates

Low molecular weight carbohydrates are important flavor precursors in coffee. By far, sucrose is the most abundant, with levels around 5.0% to 9.9% (w.b.) in Arabicas (KNOPP; BYTOF; SELMAR, 2006; KY et al., 2001; ROGERS et al., 1999; TRUGO, 1985). Fructose and glucose are also found in ranges of 0.02% to 0.40% (w.b.) and <0.01% to 0.45%(w.b.), respectively (SILWAR; LULLMAN, 1988). Traces of other low molecular weight carbohydrates, such as mannose, arabinose, rhamnose, stachyose, have also been identified in green Arabica beans (ROGERS et al., 1999; SILWAR; LULLMAN, 1988).

Sugar degradation in roasting is considerable. Sucrose degradation can reach up to 96% to 98%, depending on roast level (TRUGO; MACRAE, 1984), while fructose, glucose, and ribose disappear nearly completely (FLAMENT, 2002).

Low molecular weight carbohydrates play an important role in flavor formation. They are precursors of various aliphatic acids, which contribute to the acidity of the coffee beverage. As mentioned above, there is a direct correlation between levels of sucrose, glucose, and fructose in green coffee, and levels of formic, acetic, glycolic, and lactic acids in the resulting roasted coffee (GINZ et al., 2000). Low molecular weight carbohydrates also play necessary roles in caramelization and Maillard reactions (FLAMENT, 2002).

Associations of low molecular content have been found with fruit maturation level, processing, presence of defects, and storage level, among other factors (AVELINO et al., 2005; MAZZAFERA, 1999; TRUGO, 1985).

# 2.4 Coffee processing

Coffee processing entails drying the coffee to between 11% and 12% moisture content and removing the green seed, or bean. This can be done in several different ways.

# 2.4.1 Wet Process – Washed Coffee

The wet processing method was developed in equatorial regions with continual precipitation during the harvest period, a condition not appropriate for dry processing. In these

regions, the dry process would almost always result in coffee of inferior quality. The wet process will generally yield good quality coffee if only ripe fruit are harvested, if the skin and mucilage are properly removed, if fermentation is controlled, and if the coffee is carefully dried. Historically this method is associated with higher quality coffee and it is often used when the goal is the production of specialty coffees. This method is generally associated with selective harvesting for the production of Arabica coffee, with the exception of Brazil, Hawaii, and Australia, as well as for Robustas in several countries (BRANDO, 2009). Today, the wet process is generally carried out in three distinct ways:

## a. Fully Washed

Fully washed coffees are wet process coffees in which the pulp, <u>consisting of</u> the fruit skin (exocarp) and part of the mucilage (mesocarp), is removed mechanically, then the remaining mucilage that adheres to the parchment (endocarp), which is insoluble in water, is removed through controlled fermentation and subsequent washing. This fermentation process can be completed by simply leaving the coffee in the fermentation tank by itself (dry fermentation), by soaking the coffee in water (wet fermentation), or through mixed fermentation, which usually entails dry fermentation to rapidly acidify the coffee mass to prevent the development of yeast and molds, followed by wet fermentation (VINCENT, 1987). In all cases, the hydrolysis of pectin is caused by the biochemical action of the pectinase still present in the coffee, as well as the growth of microorganisms that produce enzymes, such as polygalacturonases and pectin lyases, that are necessary to fully depolymerize and hydrolyze the pectin present in the mucilage. Fermentation can be accelerated by different microorganisms, such as saccharomyces, which also contain pectinolytic properties (SILVA, 2015).

The duration of the fermentation process is generally between 12 and 36 hours, but will vary based on factors such as temperature, type of fermentation, maturation level of the coffee lot, height of the coffee layer, and coffee cultivar, among others. It is important to note that the wastewater from this process will have elevated levels of biochemical oxygen demand (BOD), chemical oxygen demand (COD), as well as other chemicals products, and must be treated accordingly (MATOS et al., 2014). The remaining mucilage after the fermentation process can be removed by lightly scrubbing the coffee. Historically this was performed in channels that followed the fermentation tanks, however in recent years it is becoming more common to pass the coffee through a demucilaging machine. The resulting clean parchment coffee is then dried (Figure 3.34). Fully washed coffees are the most common wet processing

method, and their flavor is generally considered to be cleaner, with a pleasant aroma, higher perceived acidity, and less body than dry process coffees (SELMAR; KLEINWÄCHTER; BYTOF, 2015; VINCENT, 1987).

#### b. Semi-Dry (Pulped Natural)

Pulped natural coffees, also referred to as semi-dry or honey(ed) coffees, are wet process coffees in which the fruit skin and part of the mucilage are removed mechanically. However, unlike wet process coffees, the remaining mucilage is not removed, but rather is dried intact with the parchment coffee. Commonly used in Brazil since the 1990's, other countries have recently adopted this method. Pulped natural coffees can be produced in various ways, and many growers experiment with different variations to positively alter the sensorial qualities of the resulting beverage, in particular by varying the amount of mucilage left on the parchment, covering the parchment coffee, or putting it in closed bags during the drying process, and altering the thickness of the drying layer. Some of these treatments alter the color of the resulting parchment coffees, which are often referred to as yellow honey, red honey, and black honey coffees accordingly. While the pulped natural method is considered here as part of the wet process, it is sometimes considered a separate category, separate from both the wet and dry methods. Although the term semi-washed may sometimes be applied to pulped natural coffees, coffees processed in this way do not normally go through a "washing" process to remove the mucilage. Furthermore, there has been a more consistent use of the term "semi-dry" in the scientific community. The flavor of pulped natural coffees is often considered to be an intermediate profile between fully washed and dry process coffees, having a "cleaner" flavor than standard naturals, but more body than most fully-washed coffees (BRANDO, 2009; TEIXIERA et al., 2005).

# c. Semi-Washed (Mechanically Demucilaged)

Semi-washed coffees are wet process coffees in which the skin and all of the mucilage are removed mechanically. They are sometimes referred to as demucilaged or mechanically demucilaged coffees. Advantages to this process are the decreased amount of wastewater generated during processing and the ease of raking and rotating the parchment coffee compared to the pulped natural method, in which the coffee clumps together. There is no consensus as to the effects of this process on flavor compared to traditional fermentation, and studies have shown mixed results (BRANDO; BRANDO, 2015; GONZALEZ-RIOS et al.,

2007). However, with increasing limitations of water availability and usage as well as wastewater disposal, this method is becoming more common.

### 2.4.2 Dry Process – Natural Coffee

The production of "natural" coffee, traditionally known as the dry method or dry process but sometimes referred to as sun-dried or unwashed coffee, is the oldest and simplest coffee processing method. It entails drying the entire coffee fruit intact and is largely used in tropical regions where the dry season coincides with the harvest period.

Traditional literature defines the dry method as the drying of all coffee fruit immediately following the harvest, with no lot separation based on maturation or coffee quality. While this is the most common way to perform the dry process, it is just one of the many processing options available and is generally the option chosen by producers with inadequate coffee processing infrastructure. In fact, all coffee, whether composed of ripe, unripe, overripe, dried coffee, or any combination thereof, is natural coffee if it was dried with its pericarp intact.

The lower quality often seen in natural coffees can be explained by two main factors: a lack of care during the harvest resulting in fruits of various qualities and maturations, and a higher risk of undesirable fermentation due to the elevated levels of sugar in the mucilage as well as the slower drying times. When only ripe fruits are selectively harvested and then carefully dried, it is possible to produce high quality dry process coffees.

In general, quality natural coffees are considered to be sweeter and fuller-bodied coffees, with flavors ranging from chocolaty and nutty to fruity, and are greatly appreciated in espresso preparation (FERNANDEZ ALDUENDA, 2015; TEIXIERA et al., 2005).

The history of the natural, or dry processing, method can be divided into three stages (FERNANDEZ ALDUENDA, 2015). The first stage began with the initial establishment of coffee as a crop and lasted until the proliferation of the wet process in the 19<sup>th</sup> Century. In this first stage, two of the most highly prized coffees in the world, Mocha from Yemen and Harrar from Ethiopia, were dry process coffees (UKERS, 1922). With the advent of the wet process, a second stage began that was characterized by the displacement of natural coffees by washed coffees. As the wet process became the norm in most producing countries, natural coffees were largely marginalized. The dry process was used mainly by growers who could not afford drying technologies or did not perform selective harvesting, as well as for fruits that were the by-product of the wet method (mostly unripe, overripe, or hollow fruits that could not be

pulped). Because of this, natural coffees were largely viewed as an inferior product to washed coffees. With the growth of the specialty coffee industry in the late 20<sup>th</sup> and early 21<sup>st</sup> Centuries, a third stage emerged, defined by renewed interest in the dry processing method. The specialty coffee movement brought a demand for high quality coffees with unique flavor profiles, as well as increased espresso consumption (the blends of which natural coffees are often a large part). Natural coffees are now produced not only in countries that traditionally produced them but also in countries throughout Central America, South America, and other regions that traditionally produced only washed coffees.

# 2.4.3 Wet hull

The wet-hulled method, called the *Giling Basah* method in Indonesia, where it is almost exclusively employed, consists of hulling the coffee when the moisture content is still high, generally between 25% and 35% (w.b.). It is customary to complete this process in two steps. As with the wet method, the fruit is initially pulped after harvesting. However, after a short period (one to two days) in which the parchment coffee is either set out to dry or soaked in buckets to remove some of the mucilage, the coffee is hulled and the naked beans are dried to completion. While there is little research and no consensus as to its impact on the flavor profile of the coffee, this process results in a deep bluish-green colored coffee.

# **2.5 Effect of processing**

It is widely accepted throughout both the coffee industry and the scientific community that different methods of processing will lead to different flavor profiles.

However, much of the classical literature delves little into the factors that are endogenous to each respective process, rather attributing the differences largely to exogenous factors, mainly the better raw material ripe fruit for wet process compared to various maturation states for dry process and the greater general care taken when processing wet process coffees (CLARKE, 1985; MACRAE, 1985; SIVETZ; FOOTE, 1963; VINCENT, 1987).

As pointed out by several authors more recently, to accurately evaluate the effects of processing, the same quality of raw material must be used for all processing techniques evaluated, and each processing method must be executed with the same care and under similar

conditions (BORÉM; ISQUIERDO; TAVEIRA, 2014; FERNANDEZ ALDUENDA, 2015; SELMAR; BYTOF; KNOPP, 2002; SELMAR; KLEINWÄCHTER; BYTOF, 2015).

Unfortunately, many of the studies that compare the wet and dry processes, especially those conducted before the year 2000, either assume that different raw materials and care in processing are inherent to the process, and thus evaluations must be performed as such, or they do not specify that the same raw materials and care were used for all methods.

A study conducted by Puerta Quintero (1996) provides a good example of the assumption that differences in raw material are inherent to certain processes. The study is an evaluation of Colombian coffee processed using the dry process, and the conclusion is that the dry process is "not recommended for Colombian coffee if the intention is to preserve quality and the traditional smoothness appreciated by consuming countries." However, in this study, on average only 42.38% of the fruit processed was ripe. This is well below the standard percentage of ripe coffee harvested using the standard harvesting techniques employed in Colombia (MONTILLA-PÉREZ et al., 2008). Perhaps even more impactful was the fact that 18% of the coffee used for this analysis comprised sweepings, coffee that has fallen from the tree onto the ground below. Sweepings are considered high risk, and it is recommended that such coffees be processed and commercialized separately (if at all) given the higher probability of mold or fermentation, as well as the coffee's low quality potential (BORÉM; ISQUIERDO; TAVEIRA, 2014).Furthermore, the study stated that during drying the coffee was rotated "occasionally." It is recommended that coffee be rotated many times a day to ensure quality (BORÉM; REINATO; ISQUIERDO, 2014; BRANDO, 2009; TEIXEIRA et al., 2005). Since the raw material and methods used do not accurately compare the dry process to the wet process, it can be reasonably inferred that the conclusion drawn by the author, that the dry process is not recommended for Colombian coffee, is not supported by the data.

In a similar study, comparing the effects of different forms of processing and mucilage removal on coffee quality, the author concluded that the form of processing that yielded the lowest evaluation scores was the dry process (PUERTA QUINTERO, 1999). However, of the two samples that were processed using the dry method, one was composed solely of unripe fruits, and it was the only sample in the experiment that was not of mature fruits. It is widely accepted that unripe fruits render a far inferior and more astringent beverage than one comprising solely ripe fruits. The other natural sample, which was composed of ripe fruits, was dried in an oven at 105°C until it reached 10% to 12% moisture content. It was not explained why the author chose to dry this sample in the oven at such a high temperature, given that all other samples were sun dried at normal ambient temperatures. This temperature

is well above the maximum temperature of 40° C recommended for drying coffee (BORÉM; REINATO; ISQUIERDO, 2014). Higher drying temperatures can compromise the physiological structure of the bean, exposing oils and other components to the effects of oxygen, thus compromising coffee quality (BORÉM et al., 2006; BORÉM; REINATO; ISQUIERDO, 2014).

For those studies that do not elucidate that the same raw material and care were used for all processing methods, one must assume that such care was not taken with the natural process, given that few studies were adamant about ensuring the same raw material was used for all processing methods. Noted exceptions to this are the research at the Universidade Federal de Lavras under Dr. Borém, and at the Technical University of Braunschweig under Dr. Selmar.

At best, one must attenuate any results that fail to bring this degree of rigor, for it cannot be known if the results were impacted by exogenous factors to the processing, or the processing itself. While these studies may provide useful information, given the changes in the coffee market mentioned previously, it is important that growers better understand more fully and objectively the flavor and chemical pathways followed with each processing technique, so that these processes may be controlled and manipulated to achieve specific chemical and sensorial profiles, not simply the results of the process as they are commonly performed in situ.

# 2.5.1 Flavor differences

Historically, language used to describe the flavor differences between wet and dry process coffees has been quite vague. In the coffee industry, it is well established that wet process coffees (washed coffees) are generally brighter and cleaner coffees than naturals, whose flavors have been described as more rustic, wild, funky, and often fruity.

In the scientific literature, many authors relate that dry process coffees have greater body than wet process coffees (GHOSH, 2014; MAZZAFERA; PURCINO, 2005; SELMAR; KLEINWÄCHTER; BYTOF, 2015; SIVETZ; FOOTE, 1963; TEIXIERA et al., 2005). Similarly there are many references to the pleasant acidity and aroma of wet process coffees (GHOSH, 2014; MAZZAFERA; PURCINO, 2005; SELMAR; KLEINWÄCHTER; BYTOF, 2015).

Fernandez Alduenda (2015) provides an extensive description of the natural flavor profile, with flavors ranging from caramel and chocolate to fresh fruit, to winey. Brando (2009) notes that the cup features of pulped naturals are closer to that of naturals in coffee originating from lower altitudes and to wet process coffees at higher altitudes; however, no specific information is given nor is a study cited.

# 2.5.2 Chemical differences

#### a. Bioactive Compounds

The literature is conflicting regarding the effect of post-harvest processing on trigonelline. Leloup et al. (2005) report a slight decrease in trigonelline when the wet process was employed, while two studies by Duarte, Pereira e Farah (2010) reported a slight increase in trigonelline when processing using the wet process compared to the dry process (2009) and to the pulped natural (semi-dry) process (2010).

Several studies show losses in caffeine content for coffee that underwent a soaking process (CHASSEVENT et al., 1969; GUYOT et al., 1995; VINCENT et al., 1977). However, more recent studies with no soaking process have shown no impact of processing on caffeine levels (BALYAYA; CLIFFORD, 1995; DUARTE; PEREIRA; FARAH, 2010; JOËT et al., 2010; LELOUP et al., 2005).

There is no consensus in the literature regarding the fate of chlorogenic acids during processing, with studies showing no change in total CGA in coffees processed using the wet process (JOËT et al., 2010), an increase in CGA levels for the dry process (BALYAYA; CLIFFORD, 1995), higher levels of CGA for fully washed coffee compared to pulped natural (DUARTE; PEREIRA; FARAH, 2010), and higher levels of CGA for fully washed coffee compared to dry process coffee (DUARTE; PEREIRA; FARAH, 2009; LELOUP et al., 2005; WOOTTON, 1973).

Though there is no consensus, the literature does point to an increase in total CGA associated with the wet process. In the study finding higher CGA levels with the dry process, the same raw material was not used for both wet process and dry process coffees (various maturation levels were used for the dry process), and as the authors point out in their conclusion, this perhaps explains the higher CGA contents in the dry process coffee (BALYAYA; CLIFFORD, 1995). Studies have shown that there is a strong influence of maturation on total CGA content (CLIFFORD; KAZI, 1987).

# b. Organic Acids

Few studies have examined the effect of processing on organic acids. Although the wet process is often associated with more acidic coffees, one of the few studies found in the literature showed that it led to decreased amounts of organic acids (LELOUP et al., 2005).

# c. Fatty Acids

Few studies have documented the effect of the post-harvest on fatty acids. Two studies have shown an increase in the content of lipids when comparing the wet and dry process (JOËT et al., 2010; LELOUP et al., 2005). Another study looked at the impact of different methods of dry processing; however, no difference were detected (JHAM et al., 2003).

### d. Low Molecular Weight Carbohydrates

There is no consensus in the literature on the impact of processing on the contents of sucrose, glucose, and fructose. Several studies report that sucrose levels are not affected by processing method (JOËT et al., 2010; KNOPP; BYTOF; SELMAR, 2006), while other studies reported that sucrose levels were lower with wet processing compared to dry processing (DUARTE; PEREIRA; FARAH, 2009) and to pulped natural processing (DUARTE; PEREIRA; FARAH, 2010).Several studies also relate that fructose and glucose levels are lower with wet processing (KNOPP; BYTOF; SELMAR, 2006; LELOUP et al., 2005), while another, with Robusta coffee, related that the dry process led to lower levels of fructose (GUYOT et al., 1995).

#### **2.5.3** Current explanations for these differences

While there is no consensus on the causes of the different flavor pathways that occur during processing, there are some theories about potential explanations.

# a. Diffusion of Compounds

In much of the historical literature, it is often implied that the aspects of the natural flavor profile, mainly enhanced body and sweetness, are the result of parts of the mucilage making their way into the bean, though the exact mechanism by which this occurs is not defined (SIVETZ; FOOTE, 1963).

One study examining the transport of sugars within the coffee plant noted that during plant development, sugar accumulation in the endocarp was not a result of diffusion from the

pericarp, but rather the transport of photosynthates from the leaves. While the perisperm does appear to play a role in this transfer, evidenced by its starch and enzyme accumulation, it does so through the plant's vascular tissue, not by serving as a transport of a large amount of sugars from the outer perisperm (GEROMEL et al., 2006).

However, by harvest time the perisperm has diminished to a thin pellicle only a few cells in thickness (DE CASTRO; MARRACCINI, 2006), and is unlikely to exhibit this same transport function of photosynthates from the leaves.

# b. Metabolic Responses (Germination and Drought Stress)

One hypothesis, put forth by a research group affiliated with the Technical University of Braunschweig (*Technische Universität Braunschweig*) and substantiated byseveral studies, is that different processing methods have different effects on metabolic reactions, in particular germinative reactions, leading to different chemical compositions and potentially accounting for some of the sensorial differences. A good overview of this hypothesis and several supporting studies can be found in Chapter 12 of Cocoa and Coffee Fermentations (SELMAR; KLEINWÄCHTER; BYTOF, 2015).

Initially classified as recalcitrant according to the seed nomenclature of *orthodox* and *recalcitrant* (ROBERTS, 1973), coffee was later redefined as exhibiting intermediate germination behavior (ELLIS; HONG; ROBERTS, 1990).Regardless of the exact nomenclature, coffee seeds, both Arabica and even more so Robusta, are not traditional orthodox seeds that undergo a period of dormancy. In fact, like recalcitrant seeds, coffee seeds are able to germinate at around 225 days after anthesis, well before the exocarp turns red or yellow, indicating fruit maturity (DE CASTRO; MARRACCINI, 2006; EIRA et al., 2006).

Although coffee seeds have the potential to germinate while still in the fruit, this does not occur. Though it is not known which inhibitive principal is active in coffee, endogenously induced germination can be suppressed either by the high osmotic potential of the fruit flesh, by germination inhibitors, or by phytohormones. As with other fruits, it is very likely that the source of germination inhibition is within the pulp. Thus, by removing the pulp, the inhibitors are removed, allowing germination to proceed. Proof of this lies in the fact that if the pericarp is not removed, the germination process will proceed only after it has extensively decomposed (SELMAR; BYTOF; KNOPP, 2002).

The active metabolism of coffee seeds during processing is demonstrated in several ways. The first if through the triphenyltetrazolium chloride (TTC) test (DIAS; DA SILVA, 1986). The second is through the loss of dry matter in wet process coffee during patio drying

(where losses earlier in the wet process may be explained by leaching, this would be a minor cause for dry matter loss during patio drying) (SELMAR; KLEINWÄCHTER; BYTOF, 2015). Next, where the content of glucose and fructose remain relatively constant in the dry process, they decrease by up to 90% in wet processing (KNOPP; BYTOF; SELMAR, 2006). Finally, the makeup of the amino acid spectrum varies considerably depending on processing type.

# 2.6 Sensory evaluation of coffee

Various systems have been developed over the years to evaluate coffee quality. Depending on their purpose, these evaluations can be either pass/fail, in which the coffee either meets or fails to meet a set standard; graded, in which the coffee is classified per preset categories; or descriptive, in which the attributes of a coffee are described to communicate a sensory flavor profile across the supply chain.

Most coffee producing countries have proprietary coffee standards, many of which contain both a physical evaluation of the green coffee bean and a sensory component to evaluate the quality of the resulting coffee beverage. In Brazil, there are two commonly employed classification systems, both of which contain a physical and sensory evaluation. For commercial grade coffee, the *Classificação Oficial Brasileira (COB)* is the most commonly used evaluation. The physical evaluation consists of counting defects, while the sensory component entails putting a coffee into one of the following categories, from best to worst cup quality: strictly soft, soft, just soft, hard, rioy, rio, and rio zona "Instrução Normativa n<sup>o</sup> 8" (BRASIL, 2003).

For higher quality coffees in Brazil, the Specialty Coffee Association of America (SCAA) protocol is often used. Like the COB, this evaluation contains both a physical aspect, which entails counting green bean defects, and a sensory evaluation. While many countries, including Brazil, often opt to conduct the physical analysis using their own country's standard or the NYBOT standard, the SCAA cupping protocol has become the most widely accepting means of evaluating specialty coffees worldwide.

Specific standards are set for the roasting and preparation of the samples, including roast time and levels, water, grind, and the physical space used for the evaluation as well as the evaluation itself. Per the protocol, the cupping is performed by completing an SCAA cupping form, which evaluates the following aspects of the coffee: Fragrance/Aroma, Flavor, Aftertaste, Acidity, Body, Balance, Uniformity, Sweetness, Defects, and Overall.

Flavor attributes are positive scores of qualities reflecting a judgment rating by the cupper. Defects are negative scores denoting unpleasant flavor sensations. The Overall score is based on the flavor experience of the individual cupper as a personal appraisal.

The quality scale for the attributes of Fragrance/Aroma, Flavor, Aftertaste, Acidity, Body, Balance, and Overall are given on a numeric scale in quarter point increments between numeric values from 6 to 10, were 6 indicates good, 7 very good, 8 excellent, 9 outstanding, and 10 is not defined but assumed to be a perfect score in the attribute. Theoretically, the scale ranges from a minimum value of 0 to a maximum value of 10, but scores below six are not included on the form as they are below specialty grade. Coffees with a total score of 80 or above are considered to be "Specialty," and coffees below 80 are "not specialty" (SCAA, 2015)

# **3. MATERIAL AND METHODS**

### **3.1 Description of coffee samples**

Samples of Yellow Catuaí (*Coffea arabica* L. cv. Catuaí Amarelo) coffee fruit were harvested in commercial crops from a plot situated 1250–1350 m.a.s.l. at Sítio Baixadão farm, outside of Cristina, Minas Gerais, Brazil (22°12'40.0"S 45°15'53.6"W).

Only fruits at peak ripeness were harvested. Pickers were instructed to harvest only yellow fruit that had turned a golden color, displayed brown spots like a ripe banana, and detached easily from the plant. Immediately after picking, a further selection was made to remove any unripe or overripe fruit, as well as any foreign material that was picked inadvertently.

The fruits were then subjected to flotation, or hydraulic separation, in which they were submerged in fresh water. This separated out the floaters, comprising coffee fruit of lower density, such as overripe, underdeveloped, and insect damaged fruit. These floaters were then discarded, leaving only the coffee that had sunk.

After hydraulic separation, the coffee was transported to the coffee processing facility at the Laboratory for the Processing of Agricultural Products (Laboratório de Processamento de Produtos Agrícolas, LLPA) at the Federal University of Lavras (Universidade Federal de Lavras, UFLA) where a further selection was completed to remove any remaining overripe, unripe, or damaged fruit, as well as any foreign material.

#### 3.2 Description of post-harvest activities and treatments

To evaluate the effect of the pericarp on the chemical composition and flavor profile of the coffee, the following actions were taken during the post-harvest. Figure 1 presents a flowchart of the post-harvest activities.

#### **3.2.1 Establishing the treatments**

After the final selection to ensure a homogeneous lot, 24 kg of coffee was separated and processed as wet process coffee (the 24 kilos comprised 3 separate 8 kg repetitions). This coffee was pulped using a Penagos DCV pulper, removing the epicarp and part of the mesocarp, and then manually washed and scrubbed with fresh water to remove any remaining mesocarp (mucilage). This coffee, pulped at 55% moisture content, was used for the sensory analysis.

The remaining fresh fruit was divided into 5 treatments with 3 repetitions of each treatment, a treatment being defined by the wet basis moisture content level at which its pericarp would be removed  $(32 \pm 2, 28 \pm 2, 22\pm 2, 18\pm 2, and 11\pm 2)$ . Each treatment contained around 8 kg of coffee fruit (approximately 12 L) and was placed into a separate tray, weighed, then set for drying. Before weighing, samples of fresh fruit were taken from each tray to determine initial moisture content. With this information, the amount of dry material could be calculated, and with that the corresponding weight at which to hull each treatment.

# 3.2.2 Drying conditions

Drying was carried out by forced convection in fixed bed dryers, composed of six square perforated trays, each with 0.35 m sides and a depth of 0.4 m, located over a plenum to ensure uniform airflow. The coffee was constantly rotated and weighed, initially every 30 minutes, then every 60 minutes once the coffees reached around 32% moisture content, and then every 2 hours once the coffees reached around 18% moisture content. The coffee rotation was done in two ways: The coffee within the trays was mixed by dumping it once into a bucket, and then back into the tray. The other rotation, completed at the same time, involved rotating the position of the tray in the drier.

Coffee in all states, including whole fruit (pericarp intact), parchment (epicarp and mesocarp removed), and green coffee beans (entire pericarp removed) was dried at the temperature of  $37 \pm 2$  °C with air flow of 24 m<sup>3</sup>min<sup>-1</sup>m<sup>-2</sup>.

Temperature was monitored using mercury thermometers at the bottom of each coffee sample mass. Temperature adjustment was completed by means of an electronic controller for each dryer.

Airflow was monitored using a vane anemometer. Once hulling began, given the drastic changes in volume caused by the removal of the pericarp, marbles were placed into trays with less volume to ensure that each tray received similar airflow as the other treatments.

#### 3.2.3 Hulling

Hulling was completed using a Carmomaq DC 1 coffee huller. Samples were quickly hulled at their corresponding moisture contents. Any remaining husk (pericarp) was removed by hand before placing the coffee back in the tray. The time taken to hull the three repetitions of each treatment, remove any remaining pericarp, weigh the coffee beans, take a sample for moisture content verification, and return the sample to the drying tray usually lasted between 30 and 60 minutes.

# 3.2.4 Storage

Once the coffee reached 11% moisture content (w.b.), it was bagged in paper bags, and those bags were put into plastic bags. After labeling the samples, they were placed in a controlled climate room, with a temperature of 10 °C and relative humidity of 60%, for a period of 30 days. After one month, part of each sample was roasted for sensory analysis, and the remaining sample was stored in a biofreezer at -80 °C until chemical analysis could be performed.

# 3.2.5 Sample preparation

Before subjecting the coffee to chemical and sensory analysis, it was size graded and sorted. The size grading entailed using metal perforated screens to separate coffee size 16 and

above, meaning that the retained coffee did not pass through a screen with round perforations with a diameter of 16/64 inch. After size grading, any abnormal beans, characterized by a deviation from the standard plane-convex shape or differences in coloration, were removed and discarded. This preparation was done to standardize the samples and minimize interference unrelated to the treatments.

Figure 1. Flowchart detailing coffee flow from harvest to final green bean coffee samples ready for analysis.



#### 3.3 Moisture content determination

The moisture content for entire fruit was determined using the Oven Method at  $105^{\circ}$ C (*Método de estufa a 105\pm1^{\circ}C* as defined in the 2009 edition of the Rules for Seeds Analysis [*Regras para Análise de Sementes*] (BRASIL, 2009). Two random samples of around 10 grams each were taken from each treatment repetition. Samples were placed into small metal containers, and these containers were randomly placed on shelves inside a forced air laboratory oven preheated to  $105\pm1^{\circ}$ C.Samples were dried at this temperature for 24 h. After 24 h, the samples were removed, lids were placed on the containers, and the containers were placed into grams vacuum desiccators until they cooled. Once cooled, samples were weighed to the nearest 0.1 mg, and differences in mass were calculated per Equation 1 to determine wet basis moisture content.

Moisture content for hulled green coffee beans was completed using ISO 6673: Green coffee – Determination of loss in mass at 105 °C (INTERNATIONAL ORGANIZATION FOR STANDARDIZATION -ISO, 2003). This procedure was the same as the method used for whole fruit detailed above, the only exception apart from the different raw material being that while a drying time of 24 hours was used for the whole fruit per the Rules for Seed Analysis, 16 hours was used for green coffee beans per ISO 6673.

Equation 1: Moisture Content Determination Moisture Content (w. b.) =  $\frac{m_1 - m_2}{m_1 - m_0} x \ 100 \ \%(1)$ 

Where:

 $m_0$  is the mass, in grams, of the dish and lid;  $m_1$  is the mass, in grams, of the dish, test portion and lid before drying  $m_2$  is the mass, in grams, of the dish, test portion and lid after drying.

# 3.4 Sensory analysis

Sensory analysis was performed by five trained coffee tasters (cuppers) using the methodology proposed by the Specialty Coffee American Association (SCAA) (SCAA, 2015). This SCAA cupping protocol was used for roasting and preparation of the coffee, as well as for the sensory evaluation.

A sample of 100 g of beans was roasted from each treatment repetition per the protocol, with roast duration between 8 and 12 minutes, and a roast level corresponding to 58

on the Agtron® "Gourmet" scale for whole beans and 63 for ground beans, with a tolerance of  $\pm 1$  point. This roast level analysis was completed visually using colored discs with corresponding Agtron® numbers as references. This reference system is part of the SCAA Roast Color Classification System kit.

After roasting, the samples were again sorted to remove any beans with yellowish coloration that differed from the roast standard. Samples were roasted the afternoon before the sensory analysis and stored in opaque metal containers overnight.

For the sensory evaluation, five cups of the three repetitions of each treatment were tasted. Per the SCAA Cupping protocol, the evaluated sensory attributes were grouped into "subjective" and "objective" categories. Subjective attributes were fragrance/aroma, flavor, acidity, body, balance, aftertaste, and overall impression. These attributes were scored by their quality on a scale of 6 to 10 points in intervals of 0.25 points (see Table 2). The objective category included uniformity, sweetness, and clean cup (absence of defects). The objective attributes were scored on a scale from 0 to 10 points, with 2 points awarded for each cup that presented satisfactory levels of a given attribute. For example, a coffee sample in which all 5 cups were clean, presented sweetness, and contained no defects would receive a total of 30 point for the objective attributes, 10 points for each objective category (2 points per cup x 5 cups per sample). For the purposes of this study, only the final score, obtained from the sum of the scores for each attribute in both categories, was considered. Table 3provides a scoring key for the final score.

Good	Very Good	Excellent	Outstanding
6.00	7.00	8.00	9.00
6.25	7.25	8.25	9.25
6.50	7.50	8.50	9.50
6.75	7.75	8.75	9.75

Table 2. SCAA scoring levels of subjective attributes, with a minimum of 6.00, a maximum of 9.75, and intervals of 0.25 points.

Final Score	Description	Grade
90–100	Outstanding	
85-89.99	Excellent	Specialty
80-84.99-89	Very Good	
< 80	Below Specialty Quality	Not Specialty

Table 3. Scoring key describing the range of coffee quality for the final, as well as coffee grade (Specialty or Not Specialty).

# 3.5 Chemical analyses

Chemical analyses were performed on the unroasted green coffee beans that had previously been dried to 11%; rested for one month in a climate controlled room (60% relative humidity and a temperature of 10 °C); hulled, sorted, and size-graded; then stored in the biofreezer where they remained until the chemical analysis. Figure 2 presents a flowchart of the sample pathway from storage in the biofreezer to each of the four chemical analyses.

Figure 2. Flowchart demonstrating the pathway of samples for each of the four chemical analyses performed (Bioactive Compounds, Organic Acids, Low Molecular Weight Carbohydrates, and Fatty Acids).



# 3.5.1 Sample preparation

In preparation for the chemical analyses, the green beans were removed from the biofreezer and ground using an Ika A11 Basic Analytical Mill. Approximately 10 ml of liquid nitrogen was used to facilitate the grinding process and inhibit oxidation of the coffee beans. These sample preparation procedures were completed in the LPPA in the Engineering Department (DEG) at UFLA. All ground samples were then freeze dried (lyophilized), then stored in a standard chest freezer at -20 °C.

#### 3.5.2 Bioactive compounds

The non-volatile compounds trigonelline, caffeine, and total chlorogenic acids were determined using High Performance Liquid Chromatography (HPLC) following a methodology proposed by Malta e Chagas (2009) adapted from Vitorino et al. (2001). Samples of 0.5 g of ground lyophilized coffee were extracted in 50 ml of boiling distilled water and then put in a water bath with boiling water for 3 min. The extract was filtered through a common paper filter, then filtered through a 0.45  $\mu$ m membrane. Determination of the compounds was done using a Shimadzu HPLC SPD-M10A Photo Diode Array Detector with a Discovery C18 HLPC Column (250 x 4.6 mm, 5 $\mu$ m), wavelength 272 *n*m. The mobile phase consisted of methanol: water:acetic acid(20:80:1), with flow of 1 ml min<sup>-1</sup>. For identification and quantitative analysis, a standardized curve was develop using standards for caffeine, trigonelline, and 5-cafeoylquinic acid.

# 3.5.3 Organic acids

Organic acids (OAs) were extracted using a method described by Jham et al. (2002), which is a slightly modified version of the method described by Van der Stegen and Van Duijn (1987). Around 2 g of ground and lyophilized coffee was mixed with water, an internal standard (glutaric acid; 15 mg) added, and the solution agitated with a magnetic stirrer for 30 min. The solution was then diluted to 10 mL, filtered, and then a 20  $\mu$ L aliquot was taken for analysis. HPLC analysis was performed using a GBC system (Victoria, Australia) model 1150 fitted with a Rheodyne injector, a Shimadzu variable UV detector, and a computer-based system to accumulate data. The analytical conditions are described in Table 4. The individual OAs in coffee samples were identified by comparing the retention times and relative (to the internal standard) retention times of the peaks with those of the standard OAs. The detection limits of the OAs (defined as two times baseline noise) were determined visually from a coffee sample. Quantification of OAs was carried out by the internal standard method by injecting known amounts of OAs and glutaric acid. Calibration curves were generated with the system software. Only linear regions of the curves were used for quantification. All samples were analyzed three times.

Column	Supelco Supelcogel-C610H (25	0 x 7,8 mm i.d.)	
Pre-column	Supelco Supelcogel-C610H (50 x 7,8 mm i.d.)		
Column temperature	40 °C		
Detector	UV operating at 210 nm		
Solvent	Water containing 1% phosphoric acid		
Flow rate program	Time (min)	Flow rate (mL/min)	
	0	0,5	
	13	0,3	
	16	0,5	
	24	0,8	

Table 4. Conditions for the HPLC analysis of organic acids in coffee bean samples.

# 3.5.4 Fatty acids

Fatty acids were extracted using a methodology adapted from Christie (1989). Around 0.25 g of ground, lyophilized coffee was weighed and placed in 1.5ml microcentrifuge tubes, then 1.0 ml of hexane was added to each tube. The tubes were then placed in an ultrasonic bath for 10 minutes to effect lipid extraction. After this, the samples were centrifuged at 6,000 rpm for 2 minutes. Next, 500 ml aliquots of each supernatant were placed in 2.0 ml cryogenic tubes and then evaporated, hydrolyzed, methylated, and analyzed by gas chromatography.

Hydrolysis was completed in a 2ml cryogenic tube by dissolving around 2 ml of oil in 100  $\mu$ l of a solution composed of 95% ethanol and 5% 1 M potassium hydroxide. After agitation in a vortex for 10 seconds, the oil was hydrolyzed in an 80 W common household microwave (Panasonic Piccolo) for 5 minutes. After cooling, 400  $\mu$ l of a 20% hydrochloric acid solution, around 20 mg of NaCl, and 600  $\mu$ l of ethyl acetate were added. After agitation in a vortex for 10 seconds and a repose period of 5 minutes, an aliquot of 300  $\mu$ L of the separated lipids was removed, put into microcentrifuge tubes, and set aside for several hours to allow for evaporation, thus obtaining the free fatty acids.

The free fatty acids were methylated with 100  $\mu$ l of BF<sub>3</sub>/methanol (14%) and heated for 10 minutes in a water bath at 80 °C. Samples were then diluted in 300  $\mu$ l of methanol.

The analysis of fatty acids was done by gas chromatography in a Shimatzu CG 2010 chromatograph (Agilent Technologies Inc., Palo Alto, CA, USA), equipped with a flame ionization detector, split injector at the rate of 1:50, and capillary column of Supelco SPTM-2560, 100 m X 0.25 mm X 0.20  $\mu$ m (Supelco Inc., Bellefonte, PA, USA). The

chromatographic conditions were an initial temperature of the column of 140 °C for 5 minutes, followed by an increase of 4 °C/minute until a temperature of 240 °C was reached and then maintained for 30 minutes, amounting to a total time of 60 minutes. The injector temperature was 260 °C and that of the detector 260 °C. The carrier gas used was helium. The fatty acids were identified by comparison with the retention times presented by the chromatographic standard SupelcoTM37 standard FAME Mix (Supelco Inc., Bellefonte, PA, USA) and expressed as percentages (%) of the total of fatty acids identified, analyzed by gas chromatography.

# 3.5.5 Low molecular weight carbohydrates

The extraction of low molecular weight carbohydrates was conducted based on(TRUGO; FARAH; CABRAL, 1995): 250 mg of each ground, lyophilized, and defatted sample was placed in a 2ml microcentrifuge tube where it was suspended in 1.0 ml of ultrapure water (18.2 M $\Omega$ ·cm at 25 °C). The tubes were placed in an ultrasonic bath heated to 60 °C for 15 minutes. A 500-µl aliquot of each extract was transferred to a 1.5ml microcentrifuge tube and these extracts were then centrifuged at 5,500 rpm for 5 minutes. The supernatant of this process was used for analysis.

Concentrations of sucrose, fructose, and glucose were determined using HPLC. Detection was performed with an electrochemical detector. Both samples and standard solutions were analyzed by injecting 20  $\mu$ l of solution, which passed through a pre-column C18 (50x4.66mm) before passing through a Water Dextropak 100 mm x 8 mm column. The mobile phase used an aqueous solution with 20 mM of NaOH at a temperature of 35 °C and a flow of 0.2 ml.min<sup>-1</sup>.

The calibration curve was determined using a stock solution containing 60.0 mg of sucrose (Sigma  $\geq$  99%) prepared in 5 ml of water. The diluted standard solutions (10% for 100% of stock solution) were used to construct the calibration curve.

Results were determined by comparing the curve peaks of the solutions with respective standards.

#### 3.6 Statistical analysis

Five post-harvest treatments were evaluated for the chemical analysis, and six postharvest treatments were evaluated for the sensory analysis, with three repetitions of each treatment. The data was submitted to analysis of variance (ANOVA) using Scott-Knot test at 5% probability. Statistical analysis was completed using SISVAR® software (FERREIRA, 2011).

# **4. RESULTS AND DISCUSSION**

Since all coffees were dried at the same temperature and all coffees were constantly rotated, any differences in the chemical or sensory profile among the coffees can be attributed to the effect of the removal of the pericarp at a given point in drying. The following are the results of the chemical analysis performed on the dried green coffee beans (11% moisture content) in which the pericarp was removed at moisture content levels (wet basis +/- 2%) 32, 28, 22, 18, and 11.

# 4.1 Bioactive compounds

The results of the bioactive compound analysis for trigonelline, caffeine, and chlorogenic acid are presented in Figure 3.

Figure 3. Percentages (dry basis) of Trigonelline (a), Caffeine (b), and Chlorogenic Acids (c) of green Arabica coffee beans with 11% moisture content (w.b.) that underwent different treatments in triplicate of pericarp removal during the drying:H32, H28, H22, H18, and H11 represent coffee samples that were hulled at 32% ± 2, 28% ± 2, 22% ± 2, 18% ± 2 and 11% ± 2 moisture content (w.b.), respectively. Bars with the same letter are not significantly different based on the Scott-Knott's test (P < 0.05).</li>



Trigonelline levels were statistically different for coffees hulled at 28%, 22%, and 18% moisture content. Neither caffeine nor total chlorogenic acid levels varied statistically.

The literature regarding the effect of processing on trigonelline is conflicting, with one study showing decreases in trigonelline with the wet process (LELOUP et al., 2005), and two showing increases with the wet process (DUARTE; PEREIRA; FARAH, 2009, 2010). However, no studies have noted increases in trigonelline levels with dry processing. This is perhaps because this increase only occurs during the drying, demonstrated here at moisture content levels 28%, 22%, and 18% (w.b.). The coffee treatment that followed the standard dry process, with the pericarp remaining intact until it was completely dry (11%), not present these elevated trigonelline levels, perhaps because of degradation.

Thus, the effect of the presence of the pericarp on trigonelline levels is a new contribution made by this study, and one with potential quality implications. As studies have shown an association of trigonelline levels with higher altitudes and higher quality (FIGUEIREDO et al., 2013; RIBEIRO et al., 2016), this provides a potential way for growers to increase quality through processing.

The removal of the pericarp at different times during drying did not cause a statistically significant change in the levels of caffeine. This result is in line with the thermostability of caffeine and concurs with several studies measuring the effects of post-harvest processing on caffeine content (BALYAYA; CLIFFORD, 1995; DUARTE; PEREIRA; FARAH, 2010; JOËT et al., 2010; LELOUP et al., 2005).

Similarly, total chlorogenic acid (CGA) levels were consistent throughout the drying process. This result concurs with one recent study (JOËT et al., 2010); yet it is a different finding from several other previous studies that have shown variances in CGA levels per processing method (BALYAYA; CLIFFORD, 1995; DUARTE; PEREIRA; FARAH, 2009, 2010; LELOUP et al., 2005). However, this does not mean that the results of this current experiment are necessarily contrary to these previous studies.

Only one study was found that showed an increase in CGA for coffee processed using the dry process. However, as noted, this study used coffee of various maturation levels for the dry process, and this can perhaps explain the increase in CGA (BALYAYA; CLIFFORD, 1995).Also, all cited studies compared processing methods, rather than the change in CGA during one process. This means that the greater levels in CGA associated with the wet process in most studies are likely a factor of their increase during the wet process, perhaps through a loss of other water-soluble components by lixiviation and fermentation, rather than their degradation during the dry process.

Another aspect to consider is the possibility that although total CGA levels were consistent, there were specific changes among different isomers. The separate behavior of classes has been observed in studies related to climate (JOËT et al., 2010) and processing (DUARTE; PEREIRA; FARAH, 2010). Since different chlorogenic classes likely have different associations with coffee quality (FARAH et al., 2006), it is recommended that further analysis be completed that examines the effect of the dry process on various chlorogenic acid classes.

# 4.2 Organic acids

The results of the analyses of organic acetic acid, citric acid, lactic acid, malic acid, quinic acid, and succinic acid are presented in Figure 4.

Figure 4. Percentages (dry basis) of Acetic Acid (a), Citric Acid (b), Lactic Acid (c), Malic Acid (d), Quinic Acid (e), and Succinic Acid (f) of green Arabica coffee beans with 11% moisture content (w.b.) that underwent different treatments in triplicate of pericarp removal during the drying: H32, H28, H22, H18, and H11 represent coffee samples that were hulled at  $32\% \pm 2$ ,  $28\% \pm 2$ ,  $22\% \pm 2$ ,  $18\% \pm 2$  and  $11\% \pm 2$  moisture content (w.b.), respectively. Bars with the same letter are not significantly different based on the Scott-Knott's test (P < 0.05).



Figure 4 shows that the levels of acetic acid, citric acid, lactic acid, and succinic acid remained constant throughout the drying process. However, the levels of malic acid and quinic acid both increased.

The increase in levels of quinic acid can perhaps be explained by degradation of chlorogenic acid during the drying process. Although no statistically significant change was noted in chlorogenic acid over the same period, quinic acid is a byproduct of CGA degradation. Given the large relative difference in the quantities of these substances, it is possible that the CGA degradation caused a statistically significant increase in quinic acid without changing significantly itself.

Malic acid is mostly biosynthesized, and no accounts were found of its generation after the coffee was harvested. As it is generally considered to be a pleasant flavor in coffee (LINGLE, 2011), the generation of malic acid in the post-harvest should be further examined.

# 4.3 Fatty acids

The results of the fatty acid analysis for arachidic acid, behenic acid, erucic acid, linoleic acid, linolenic acid, myristic acid, oleic acid, palmitic acid, and stearic acid are presented in Figure 5. Figure 5. Percentages of Arachidic Acid (a), Behenic Acid (b), Erucic Acid (c), Linoleic Acid (d), Linolenic Acid (e), Myristic Acid (f), Oleic Acid (g), Palmitic Acid (h), and Stearic Acid (i) of green Arabica coffee beans with 11% moisture content (w.b.) that underwent different treatments in triplicate of pericarp removal during the drying: H32, H28, H22, H18, and H11 represent coffee samples that were hulled at 32% ± 2, 28% ± 2, 22% ± 2, 18% ± 2 and 11% ± 2 moisture content (w.b.), respectively. Bars with the same letter are not significantly different based on the Scott-Knott's test (P < 0.05).</p>



Figure 5 shows that there was no statistically significant alteration in levels of any of the fatty acids during processing.

This result is consistent with other studies examining fatty acids during the dry process (JHAM et al., 2001, 2003). While other studies have shown higher levels of fatty acids in wet process coffee compared to the dry process, these studies did not show that this was due to a change in the fatty acids levels that occurred as a result of the dry process (JOËT et al., 2010; LELOUP et al., 2005).

# 4.4 Low molecular weight carbohydrates

The results of the low molecular weight carbohydrate analyses for sugar, glucose, and fructose are presented in Figure 6.

Figure 6. Percentages (dry basis) of Sucrose (a), Glucose (b), and Fructose (c) of green Arabica coffee beans with 11% moisture content (w.b.) that underwent different treatments in triplicate of pericarp removal during the drying: H32, H28, H22, H18, and H11 represent coffee samples that were hulled at 32% ± 2, 28% ± 2, 22% ± 2, 18% ± 2 and 11% ± 2 moisture content (w.b.), respectively. Bars with the same letter are not significantly different based on the Scott-Knott's test (P < 0.05).</li>



Figure 6 shows that sucrose, glucose, and fructose levels were not statistically different in this study. These results concur with a previous study that determined that while the contents of fructose and glucose decreased with wet processing, they remained unchanged in dry processing (KNOPP; BYTOF; SELMAR, 2006). This result provides further evidence against the commonly held belief that dry processed coffees are sweeter because of sugar migration to the endocarp from the pericarp during processing.

# 4.5 Sensory analysis

The results of the sensory analysis are presented in Figure 7. Different from the chemical analysis, the treatment of a coffee pulped at the onset of processing, with a moisture content of 55% (w.b.), was included in this analysis (H55).

Figure 7. Overall cupping scores for roasting Arabica coffee beans dried to 11% moisture content (w.b.) with different treatments in triplicate of pericarp removal during drying. Roasting, coffee preparation, and sensory analysis were completed using Specialty Coffee Association (SCAA) Cupping Protocols. Bars with the same letter are not significantly different based on the Scott-Knott's test (P < 0.05).



As shown in Figure 7, there was no statistically significant difference between the overall scores from the cuppers for the various treatments.

While no statistical difference was noted in the overall score of the coffee, the description of the flavors of the treatments varied, though not in a systemic way. The SCAA methodology used for the sensory analysis is a method of descriptive analysis. Two coffees that have the same numerical score, can nonetheless present varying flavor profiles. It is recommended that further research be done to explore the possibility of grouping flavor descriptors to examine if different flavor profiles occur with different treatments.

# **5 CONCLUSION**

Given the results of this study, it can be stated that the removal of the pericarp at different points during the drying process corresponds with different chemical compositions of the resulting dried green coffee beans, as well as differences in the descriptive sensory analysis of those beans when they are roasted.

The presence of the pericarp affected the levels of trigonelline, malic acid, and quinic acid. Levels of caffeine, CGA, fatty acids, most organic acids (acetic, citric, lactic, and succinic), as well as glucose, sucrose, and fructose levels were unaffected by the presence of the pericarp during drying.

Understanding how processing affects the chemical composition of the coffee bean, as well as how this chemical composition relates to different flavor profiles in the cup, is a complex endeavor. While components here are presented individually, it is rather their combination that influences both roasting transformation and the final flavor profile of the coffee beverage. Further studies in processing should examine the different chemical profiles that are associated with different processing methods, and how these profiles, in turn, lead to different flavor profiles in the cup.

# WORKS CITED

ALCÁZAR, A. et al. Ion chromatographic determination of some organic acids, chloride and phosphate in coffee and tea. **Talanta**, London, v. 61, n. 2, p. 95–101, 2003.

ARORA, D. S.; OHLAN, D. In vitro studies on antifungal activity of tea (Camellia sinensis) and coffee (Coffea arabica) against wood-rotting fungi. **J. Journal of Basic Microbiology**, Weinheim, v. 37, n. 3, p. 159–165, 1997.

ASHIHARA, H.; SANO, H.; CROZIER, A. Caffeine and related purine alkaloids: Biosynthesis, catabolism, function and genetic engineering. **Phytochemistry**, New York, v. 69, p. 841–856, 2008.

AVELINO, J. et al. Effects of slope exposure, altitude and yield on coffee quality in two altitude terroirs of Costa Rica, Orosi and Santa María de Dota. Journal of the Science of Food and Agriculture, London, v. 85, n. 11, p. 1869–1876, 2005.

BALYAYA, K. J.; CLIFFORD, M. N. Chlorogenic acids and caffeine content of monsooned Indian Arabica and Robusta coffees compared with wet and dry processed coffees from the same geographic area. In: COLLOQUE SCIENTIFIQUE INTERNATIONAL SUR LE CAFÉ, 16., 1995, Kyoto.**Proceedings**... Paris: ASIC, 1995. p. 316-325.

BALZER, H. H. Acids in cofee. In: CLARKE, R. J.; VITZTHUM, O. G. (Ed.). Coffee recent developments. Oxford: Blackwell Science, 2001. p. 18–32.

BELITZ, H. D.; GROSCH, W.; SCHIEBERLE, P. Food chemistry. 4th ed. Berlin: Springer-Verlag, 2009.

BERTRAND, B. et al. Comparison of the effectiveness of fatty acids, chlorogenic acids, and elements for the chemometric discrimination of coffee (Coffea arabica L.) varieties and growing origins. Journal of Agricultural and Food Chemistry, Easton, v. 56, n. 6, p. 2273–2280, 2008.

BORÉM, F. M. et al. Qualidade do café submetido a diferentes temperaturas. **Coffee Science**, Lavras, v. 1, n. 1, p. 55–63, 2006.

BORÉM, F. M.; GARCIA SALVA, T. J.; AMARAL DA SILVA, E. A. Anatomy and chemical composition of the coffee fruit and seed. In: BORÉM, F. M. (Ed.). Handbook ofcoffee Post-Harvest Technology. Norcross: Gin, 2014. p. 1–10.

BORÉM, F. M.; ISQUIERDO, É. P.; TAVEIRA, J. H. S. Coffee processing. In: BORÉM, F. M. (Ed.). Handbook of coffee Post-Harvest Technology. Norcross: Gin, 2014. p. 49–68.

BORÉM, F. M.; REINATO, C. H. R.; ISQUIERDO, É. P. Coffee drying. In: BORÉM, F. M. (Ed.).**Handbook of coffee Post-Harvest Technology**. Norcross: Gin, 2014. p. 97–118.

BRANDO, C. H. J.; BRANDO, M. F. P. Methods of coffee fermentation and drying. In: SCHWAN, R.; FLEET, G. H. (Ed.). Cocoa and coffee fermentations. Boca Raton: CRC, 2015. p. 367–396.

BRANDO, C. H. J. Harvesting and green coffee processing. In: WINTGENS, J. N. (Ed.).**Coffee:** growing, processing, sustainable production. 2nd ed. Weinheim: Wiley-VCH, 2009. p. 610–723.

BRASIL. Ministério da Agricultura, Pecuária e Abastecimento. **Instrução Normativa nº 8**, **de 11 de junho de 2003**. Disponível em: <a href="http://www.abic.com.br/publique/media/NMQ\_LEGISLAcaO\_IN8.pdf">http://www.abic.com.br/publique/media/NMQ\_LEGISLAcaO\_IN8.pdf</a>>. Acesso em: 21 dez. 2016.

BRASIL. Ministério da Agricultura Pecuária e Abastecimento. **Regras para análise de sementes**. Brasília, 2009. Disponível em: <a href="http://www.agricultura.gov.br/assuntos/insumos-agropecuarios/arquivos-publicacoes-insumos/2946\_regras\_analise\_\_sementes.pdf">http://www.agricultura.gov.br/assuntos/insumos-agropecuarios/arquivos-publicacoes-insumos/2946\_regras\_analise\_\_sementes.pdf</a>>. Acesso em: 22 dez. 2016.

CARVALHO, C. H. S. et al. Cultivares de café Arábica de porte baixo. In: CARVALHO, C. H. S. (Ed.). **Cultivares do café**: origem, características e recomendações.Brasilia: Embrapa Café, 2008. p. 157–226.

CASAL, S.; BEATRIZ OLIVEIRA, M.; FERREIRA, M. A. HPLC/diode-array applied to the thermal degradation of trigonelline, nicotinic acid and caffeine in coffee. **Food Chemistry**, London, v. 68, n. 4, p. 481–485, 2000.

CHASSEVENT, F. et al. Étude de relations éventuelles gustatives ou chimique en function de la préparation du café. In:COLLOQUE INTERNATIONAL SUR LA CHIMIE DES CAFÉS, 4., 1969, Amsterdam.**Proceedings**...Amsterdam: ASIC, 1969.

CHRISTIE, W. W. Gas chromatography and lipids: a practical guide.Oxford: Pergamon, 1989.

CLARKE, R. J. Green coffee processing. In: CLIFFORD, M. N.; WILLSON, K. C. (Ed.).**COFFEE botany, biochemistry and production**. Wesport: AVI, 1985. p. 230–251.

CLIFFORD, M. N. Chlorogenic acids. In: CLARKE, R. J.; MACRAE, R. (Ed.). Coffee. Essex: Elsevier Science, 1985. p. 153–202.

CLIFFORD, M. N. et al. Characterization by LC-MSn of four new classes of chlorogenic acids in green coffee beans: Dimethoxycinnamoylquinic acids, diferuloylquinic acids, caffeoyl-dimethoxycinnamoylquinic acids, and feruloyl- dimethoxycinnamoylquinic acids. **Journal of Agricultural and Food Chemistry**, Easton, v. 54, n. 6, p. 1957–1969, 2006.

CLIFFORD, M. N.; KAZI, T. The influence of coffee bean maturity on the content of chlorogenic acids, caffeine and trigonelline. **Food Chemistry**, London, v. 26, p. 59–69, 1987.

COFFEE QUALITY INSTITUTE. Annual report. Aliso Viejo, 2015.

COFFEE QUAILTY INSTITUTE. Annual report. Long Beach, 2005.

DART, S. K.; NURSTEN, H. E. Volatile compounds. In: CLARKE, R. J.; MACRAE, R. (Ed.). **Coffee.** Essex: Elsevier, 1985. p. 223–265.

DE CASTRO, R. D.; MARRACCINI, P. Cytology, biochemistry and molecular changes during coffee fruit development. **Brazilian Journal of Plant Physiology,** Londrina, v. 18, n. 1, p. 175–199, 2006.

DESSALEGN, Y. et al. Genetic diversity and correlation of bean caffeine content with cup quality and green bean physical characteristics in coffee (Coffea arabica L.). Journal of the Science of Food and Agriculture, London, v. 88, n. 10, p. 1726–1730, 2008.

DIAS, M. C. L.; DA SILVA, W. R. The tetrazolium test for viability evaluation of coffee bean seed. **Pesquisa Agropecuaria Brasileira**, Brasília, v. 21, p. 1139–1145, 1986.

DUARTE, G. S.; PEREIRA, A. A.; FARAH, A. Chemical composition of Brazilian green coffee seeds processed by dry and wet Post-Harvesting methods.In:COLLOQUE SCIENTIFIQUE INTERNATIONAL SUR LE CAFÉ, 22.,2009, Campinas.Proceedings...Campinas: ASOC, 2009. 1 CD-ROM.

DUARTE, G. S.; PEREIRA, A. A.; FARAH, A. Chlorogenic acids and other relevant compounds in Brazilian coffees processed by semi-dry and wet post-harvesting methods. **Food Chemistry**, London, v. 118, n. 3, p. 851-855, 2010.

DUSSERT, S. et al. Oxidative stress, phospholipid loss and lipid hydrolysis during drying and storage of intermediate seeds. **Physiologia Plantarum**, Copenhagen, v. 127, n. 2, p. 192–204, 2006.

EIRA, M. T. S. et al. Coffee seed physiology. **Brazilian Journal of Plant Physiology**, Londrina, v.18, n.1, p.149-163, jan./mar. 2006

ELLIS, R. H.; HONG, T. D.; ROBERTS, E. H. An intermediate category of seed storage behaviour? **Journal of Experimental Botany**, Oxford, v. 41, n. 9, p. 1167–1174, 1990.

ELLIS, R. H.; HONG, T. D.; ROBERTS, E. H. An intermediate category of seed storage behaviour?**Journal of Experimental Botany**, Oxford, v. 42, n. 5, p. 653–657, 1991.

FARAH, A. Coffee as a functional beverage. **Agro Food Industry Hi-Tech**, Milano, v. 20, n. 6, p. 36–39, 2009.

FARAH, A. et al. Correlation between cup quality and chemical attributes of Brazilian coffee. **Food Chemistry**, London, v. 2, n. 98, p. 373-380, 2006.

FERNANDEZ ALDUENDA, M. R. Effect of processing on the flavour character of Arabica Natural Coffee. 2015. 266 p.Thesis (Doctor of Philosophy) - University of Otago, Dunedin, 2015.

FERREIRA, D. F. Sisvar: a computer statistical analysis system. **Ciencia e Agrotecnologia**, Lavras, v. 35, n. 6, p. 1039–1042, 2011.

FIGUEIREDO, L. P. et al. The potential for high quality Bourbon Coffees from different environments. **Journal of Agricultural Science**, Cambridge, v. 5, n. 10, p. 87–98, 2013.

FLAMENT, I. Coffee flavor chemistry. West Sussex: J. Wiley & Sons, 2002.

FOLSTAR, P. Lipids. In: CLARKE, R. J.; MACRAE, R. (Ed.). Coffee.Essex: Elsevier, 1985. p. 203–222.

FRANCA, A. S. et al. Physical and chemical attributes of defective crude and roasted coffee beans. **Food Chemistry**, London, v. 90, n. 1/2, p. 89–94, 2005.

FRANCA, A. S.; MENDONÇA, J. C. F.; OLIVEIRA, S. D. Composition of green and roasted coffees of different cup qualities. **LWT - Food Science and Technology**, London, v. 38, n. 7, p. 709–715, 2005.

GEROMEL, C. et al. Biochemical and genomic analysis of sucrose metabolism during coffee (Coffea arabica) fruit development. **Journal of Experimental Botany**, Oxford, v. 57, n. 12, p. 3243–3258, 2006.

GHOSH, P. Processing and drying of coffee: areview. **International Journal of Engineering Research & Technology**, Perlis, v. 3, n. 12, p. 784–794, 2014.

GINZ, M. et al. Formation of aliphatic acids by carbohydrate degradation during roasting of coffee. **European Food Research and Technology**, Berlin, v. 211, p. 404–410, 2000.

GONZALEZ-RIOS, O. et al. Impact of "ecological" post-harvest processing on coffee aroma: II. Roasted coffee. **Journal of Food Composition and Analysis**, San Diego, v. 20, p. 297-307, 2007.

GUYOT, B. et al. Influence du mode de préparation du café vert robusta sur sa composition chimique et ses qualités organoleptiques. In: COLLOQUE SCIENTIFIQUE INTERNATIONAL SUR LE CAFÉ, 16., 1995, Kyoto.**Annales**...Paris: ASIC, 1995.

HOLLINGSWORTH, R. G.; ARMSTRONG, J. W.; CAMPBELL, E. Caffeine as a novel toxicant for slugs and snails. **Annals of Applied Biology,** London, v. 142, n. 1, p. 91–97, 2003.

ICO INDICATOR PRICE.Disponível em: <a href="http://www.ico.org/prices/p1-June2016.pdf">http://www.ico.org/prices/p1-June2016.pdf</a>>. Acesso em: 22 nov. 2016.

INTERNATIONAL ORGANIZATION FOR STANDARDIZATION. **ISO 6673: 2003:** green coffee - determination of loss in mass at 105 °C. Geneva, 2003.

JHAM, G. N. et al. Comparison of GC and HPLC for the Quantification of organic acids in coffee. **Phytochemical Analysis**, Chichester, v. 13, n. 2, p. 99–104, 2002.

JHAM, G. N. et al. Determination of the triacylglycerol composition of coffee beans by reverse-phase high-performance liquid chromatography. **Phytochemical Analysis**, Chichester, v. 14, n. 5, p. 310–314, 2003.

JHAM, G. N. et al. Lipid classes and triacylglycerols in coffee samples from Brazil: Effects of coffee type and drying procedures. **Food Research International**, Essex, v. 34, n. 2–3, p. 111–115, 2001.

JOËT, T. et al. Influence of environmental factors, wet processing and their interactions on the biochemical composition of green Arabica coffee beans. **Food Chemistry**, London, v. 118, n. 3, p. 693-701,2010.

KNOPP, S.; BYTOF, G.; SELMAR, D. Influence of processing on the content of sugars in green Arabica coffee beans. **European Food Research and Technology**, Berlin, v. 223, p. 195-201, 2006.

KY, C. L. et al. Caffeine, trigonelline, chlorogenic acids and sucrose diversity in wild Coffea arabica L. and C. canephora P. accessions. **Food Chemistry**, London, v. 75, n. 2, p. 223-230, 2001.

LELOUP, V. et al. Impact of wet and dry process on green coffee composition and sensory characteristics.In: INTERNATIONAL CONFERENCE ON COFFEE SCIENCE, 20., 2004, Bangalore. **Proceedings**... Bangalore: ASIC, 2005. 1 CD-ROM.

LINGLE, T. **The coffee cupper's handbook**. 4th ed. Long Beach: Specialty Coffee Association of America, 2011.

MACRAE, R. Nitrogenous components. In: CLARKE, R. J.; MACRAE, R. (Ed.). Coffee. Essex: Elsevier, 1985. p. 115–152.

MALTA, M. R.; CHAGAS, S. J. R. Avaliacao de compostos nao-volateis em diferentes cultivares de cafeeiro produzidas na regiao sul de Minas Gerais. Acta Scientiarum. Agronomy, Maringá, v. 31, n. 1, p. 57–61, 2009.

MATOS, A. et al. Treatment of residues in post harvest coffee. In: BORÉM, F. M. (Ed.).**Handbook of coffee post-harvest technology**. Norcross: Gin, 2014.p. 49–68.

MAZZAFERA, P. Chemical composition of defective coffee beans. Food Chemistry, London, v. 64, n. 4, p. 547–554, 1999.

MAZZAFERA, P.; GONCALVES, K. V. Nitrogen compounds in the xylem sap of coffee. **Phytochemistry**, Neew York, v. 50, p. 383-386, 1998.

MAZZAFERA, P.; PURCINO, R. Post harvest processing methods and physiological alterations in the coffee fruit.In: COLLOQUE SCIENTIFIQUE INTERNATIONAL SUR LE CAFÉ, 20., 2005, Bangalore. **Proceedings**...Bangalore: ASIC, 2005. 1 CD-ROM.

MAZZAFERA, P. Trigonelline in coffee. **Phytochemistry**, New York, v. 30, n. 7, p. 2309–2310, 1991.

MENDES, A. N. G. et al. Mutantes de coffea arabica L. e cultivares de import:ânica histórica. In: CARVALHO, C. H. S. DE (Ed.).**Cultivares de café origem, características e recomendações**. Brasilia: Embrapa Café, 2008. p. 281–298.

MONTILLA-PÉREZ, J. et al. Propriedades físicas y factores de conversión del café en el proceso de beneficio. **Avances Técnicos Cenicafé**, Chinchiná, v. 370, p. 1-8, abr. 2008.

PERRONE, D. et al. Comprehensive analysis of major and minor chlorogenic acids and lactones in economically relevant Brazilian coffee cultivars. **Food Chemistry**, London, v. 106, n. 2, p. 859–867, 2008.

PUERTA-QUINTERO, G. I. Evaluación de la calidade del café colombiano procesado por via seca. **Cenicafé**, Chinchiná, v. 47, n. 2, p. 85–90, 1996.

PUERTA QUINTERO, G. I. Influencia del proceso de beneficio en la calidad del cafe. **Cenicafé**, Chinchiná, v. 50, n. 1, p. 78–88, 1999.

RAUT, J. S. et al. Inhibition of planktonic and biofilm growth of Candida albicans reveals novel antifungal activity of caffeine. **Journal of Medicinal Plants Research**, Boulder, v. 7, n. 13, p. 777–782, 2013.

RIBEIRO, D. E. et al. Interaction of genotype, environment and processing in the chemical composition expression and sensorial quality of Arabica coffee. **African Journal of Agricultural Research**, Nairobi, v. 11, n. 27, p. 2412–2422, 2016.

RIBEIRO, D. E. Interação genótipo e ambiente na composição química e qualidade sensorial de cafés especiais em diferentes formas de processamento. 2013. 62 p. Dissertação (Mestrado em Engenharia Agrícola) - Universidade Federal de Lavras, Lavras, 2013. Disponível em: <a href="http://repositorio.ufla.br/jspui/handle/1/766">http://repositorio.ufla.br/jspui/handle/1/766</a>>. Acesso em: 2 jul. 2015

RIZVI, S. J. H.; PANDEY, S. K.; MATHUR, S. N. 1,3,7-Trimethylxanthine, a new chemosterilant for stored grain pest, Callosobruchus chinensis (L.). Journal of Applied Entomology, Berlin, v. 90, n. 1/5, p. 378–381, 1980.

ROBERTS, E. H. Predicting the storage life of seeds. Seed Science and Technology, Zurich, v. 1, p. 499–514, 1973.

ROGERS, W. J. et al. Changes to the content of sugars, sugar alcohols, myo-inositol, carboxylic acids and inorganic anions in developing grains from different varieties of Robusta (Coffea canephora) and Arabica (C. arabica) coffees. **Plant Science**, Limerick, v. 149, p. 115-123, 1999.

SELMAR, D.; BYTOF, G.; KNOPP, S. E. New aspects of coffee processing: the relation between seed germination and coffee quality. In: COLLOQUE SCIENTIFIQUE INTERNATIONAL SUR LE CAFÉ, 19., 2002, Trieste.**Proceedings**...Trieste: ASIC, 2002. 1 CD-ROM.

SELMAR, D.; KLEINWÄCHTER, M.; BYTOF, G. Metabolic responses of coffee beans during processing and their impact on coffee flavor. In: SCHWAN, R. F.; FLEET, G. H. (Ed.). **Cocoa and coffee fermentations**. Boca Raton: CRC, 2015. p. 431–476.

SILVA, C. F. Microbial activity during coffee fermentation. In: SCHWAN, R. F.; FLEET, G. H. (Ed.). **Cocoa and coffee fermentations**. Boca Raton: CRC, 2015. p. 397–430. SILVAROLLA, M. B.; MAZZAFERA, P.; FAZUOLI, L. C. A naturally decaffeinated abrabica coffee. **Nature**, London, v. 429, n. 826, p. 826, 2004.

SILWAR, R.; LULLMAN, C. The determination of mono- and disaccharides in green Arabica and Robusta coffees using high performance liquid chromatography. **Cafe, Cacao, Thé**, São Paulo, v. 32, p. 319–322, 1988.

SIVETZ, M.; FOOTE, H. E. Coffee processing technology. Westport: AVI, 1963.

SPECIALTY COFFEE ASSOCIATION OF AMERICA. **SCAA Protocols:** cupping protocol. Santa Ana, 2015. Disponível em: <a href="http://www.scaa.org/?page=resources&d=cupping-protocols">http://www.scaa.org/?page=resources&d=cupping-protocols</a>. Acesso em: 22 dez. 2016.

SPEER, K.; KOHLLING-SPEER, I. The lipid fraction of the coffee bean. **Brazilian Journal** of Plant Physiology, Londrina, v. 18, n. 1, p. 201–216, 2006.

STEIMAN, S. What is specialty coffee. In: THURSTON, R. W.; MORRIS, J.; STEIMAN, S. (Ed.). **Coffee A comprehensive guide to the bean, the beverage, and the industry**. Lanham: Rowman & Littlefield, 2013. p. 102–105.

TEIXIERA, A. A. et al. Processing of the Harvest. In: ILLY, A.; VIANI, R. (Ed.). Espresso coffee the science of quality. 2nd ed. San Diego: Elsevier, 2005. p. 91–96.

THE COFFEE exporter's guide. 3rd ed. Geneva: International Trade Centre, 2011. 247 p.

TRUGO, L. C. Carbohydrates. In: CLARKE, R. J.; MACRAE, R. (Ed.). Coffee. Essex: Elsevier, 1985. p. 83–152.

TRUGO, L. C.; FARAH, A.; CABRAL, L. Oligosaccharide distribution in Brazilian soya bean cultivars. **Food Chemistry**, London, v. 52, n. 4, p. 385–387, 1995.

TRUGO, L. C.; MACRAE, R. A Study of the Effect of Roasting on the Chlorogenic Acid Composition of Coffee Using HPLC. **Food Chemistry**, London, v. 15, n. 3, p. 219–227, 1984.

UKERS, W. H. All about coffee. New York: The Tea and Coffee Trade Journal Company, 1922.

VAN DER STEGEN, G. H. D.; DUIJN, J. Analysis of normal organic acids in coffee. In: COLLOQUE SCIENTIFIQUE INTERNATIONAL SUR LE CAFÉ, 12., 1987, Montreaux. Annales...Paris: ASIC, 1987. p. 238-246.

VIANI, R.; HORMAN, I. Thermal behavior of trigonelline. Journal of Food Science, Champaign, v. 39, n. 6, p. 1216–1217, 1974.

VILLARREAL, D. et al. Genotypic and environmental effects on coffee (Coffea Arabica L.) bean fatty acid profile: impact on variety and origin chemometric determination. Journal of Agricultural and Food Chemistry, Easton, v. 57, n. 23, p. 11321–11327, 2009.

VINCENT, J. C. et al. Influence de différents traintements technologiques sur le caractéristiques chimiques et organoleptiques des cafés Robusta et Arabusta. In: INTERNATIONAL COLLOQIUM ON THE CHEMISTRY OF COFFEE, 8., 1977, Paris. Annales...Paris: ASIC, 1977. 1 CD-ROM.

VINCENT, J. C. Green coffee processing. In: CLARKE, R. J.; MACRAE, R. (Ed.). Coffee. Dordrecht: Springer, 1987. p. 1.

VITORINO, M. D. et al. Metodologia de obtencao de extrato de cafe visando a dosagem de compostos nao volateis. **Revista Brasileira de Armazenamento**, Viçosa, MG, v. 26, n. 3, p. 17–24, 2001.

WOOTTON, A. E. **The dry matter loss from parchment coffee during field processing**. In:COLLOQUE INTERNATIONAL SUR LA CHIMIE DES CAFÉS VERTS, TORRÉFIÉS ET LEURS DÉRIVÉS, 5., 1973, Lisbonne. **Annales**...Paris: ASIC, 1973. p. 316-324.