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Chemical Composition and Sensory Quality of Coffee Fruits at Different Stages of Maturity

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Abstract: The configuration of the sensory quality of coffee begins in the tree, where chemical precursors are deposited and formed in the seeds as the fruits develop. Fruit within the range classified as mature can have a wide range of properties. This study evaluated three degrees of maturity and established their chemical and sensory characteristics using analytical techniques such as liquid and gas chromatography. The maturity states evaluated did not show differences in organic acids, free fatty acids, lipids, total chlorogenic acids, proteins, alkaloids or sucrose. Fructose and glucose showed differences with respect to the degree of maturity, with higher values associated with more developed states. The analysis of variance did not show a significant effect on sensory attributes or sensory quality. The chromatic coordinate a* of the CIEL*a*b* scale reached a maximum value of 25.16, and the evaluated states were different from each other.

Keywords: coffee; quality; maturity; glucose; fructose; sensory

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1. Introduction

The fruit of *Coffea arabica* L. is a drupoid and reaches its maximum development between 220 and 240 days after anthesis; it is initially green and later develops yellow or red tones depending on the genotype [1]. It consists of a well-developed pericarp or cortex with three characteristic tissues: the exocarp, mesocarp and endocarp [2,3]. The endosperm consists of irregularly shaped cells with very thin cell walls and a large number of amyloplasts inside. Most of the endosperm is composed of parenchymal storage cells that accumulate materials, both in the cytoplasm and in the walls [3]. Knowledge of the different structures that make up the fruit, the relationships and biochemical pathways of metabolic flow between tissues and the accumulation of materials within the endosperm is fundamental to identify the objectives for improving the quality of the mature bean [4,5].

Fruit growth can be described by a logistic function in a sigmoidal curve: an initial exponential stage of slow growth, a linear stage of accelerated growth and a final stage of stabilization. Subsequently, a decrease stage is observed, associated with degradative and postmaturation processes [1]. The last phase is characterized by weight losses associated with dehydration, microorganism attack and increased fruit respiration [1,6]. During the initial development phase of the coffee fruit, the water content approaches 80% (fresh weight); subsequently, the water content decreases, related to the increase in dry weight due to the accumulation of dry matter of the seed during maturation. The variation in water content between fruit components seems to be related to the coffee species, variety and climatic conditions during development [5]. The water content of the seed reaches approximately 50% at the end of ripening, while that of the fruit remains at approximately 70%, due to the mucilaginous mesocarp characteristic of mature fruits [5]. The pectin content of this tissue is characterized by its high water retention capacity. The amount of

mucilage with respect to the weight of the fruit varies with maturity; mature and fresh fruits contain 10.4% mucilage on average [7].

Coffee fruits turn red when ripe due to the replacement of chlorophyll in the exocarp by red flavonoid pigments. The color of the cherry is a good marker of maturation and is correlated with the development of high-quality flavors in the coffee beverage [8]. The chlorophyll content varies with the species and decreases without ever reaching zero, while the contents of carotenoids and anthocyanins increase [1]. Coffee fruits are usually harvested according to the color of the fruit. Selective harvesting is performed manually by selecting ripe fruits in a timely manner and avoiding immature, half-ripe and overripe beans; this method is used to harvest coffee in approximately 10 to 15 countries per year in equatorial regions [9]. Green, half-ripe, ripe, overripe and dry fruits have specific characteristics that ultimately affect the quantity and quality of the final product [10,11]. Some authors have measured the color of fruits using different models and color methods: the Royal Horticultural Society color chart; the Pantone color chart; and RGB, HSV and YIQ color spaces [12]. Color is a perceived characteristic, and, hence, the use of different scales to help in color interpretation is becoming more common; these scales express color in easily reproducible numerical terms that correspond to reality. The L*a*b* color space, also referred to as CIELAB, is one of the most commonly used color scales to evaluate the color of an object because it correlates numerical values with human visual perception. This scale was designed according to color theory that establishes the opposites of two colors. In this case, for the chromatic coordinate a*, an object cannot be red and green at the same time, which is very useful for the definition of the maturation stage of coffee fruit. According to the CIEL*a*b* scale, the luminosity of the coffee fruit varies with the stage of development, reaching its highest intensity in fruits at 31 weeks of development. Through the chromatic coordinates a* and b* and various light wavelengths reflected by the epidermis, coffee fruits at 31, 32, 33 and 34 weeks of development can be differentiated [12].

Bean filling occurs through a sequential growth pattern associated with the biosynthesis of the compounds that are present in the mature bean. Some compounds, such as proteins and lipids, are synthesized in the mature bean, that is, the endosperm. Other compounds, such as caffeine, are synthesized in chlorophyll tissues, mainly in the leaves but also in the pericarp. Such compounds synthesized in the chlorophyll tissues are transported to the bean, which implies not only transport in vascular vessels but also an important phenomenon of exchange between the different tissues of the fruit [6].

Carbohydrates play an important role in the maturation process through depolymerization, which leads to a decrease in molecular size. The main classes of cell wall polysaccharides that undergo modifications during maturation are starch, pectin, cellulose and hemicellulose [13]. Most of the sugars present in the fruit are sucrose, fructose and glucose, which are found in higher concentrations in the perisperm of mature coffee beans. Higher levels of sucrose in the endosperm are correlated with higher levels of glucose and fructose in the perisperm tissue. Organic acids such as citric and malic acids dominate in mature bean, with higher concentrations in Arabica than in Canephora [14]. Ref. [6] showed that there are significant differences in protein composition during maturation.

Ref. [15] discovered that the content of chlorogenic acid (CGA) in coffee beans decreases with the maturation of the coffee fruit and that there is a difference between ripe fruit (pink) and fully ripe fruit (red). Ref. [16] worked with fruits of two varieties of Arabica, Typica and Catuai, which were harvested at three different stages of maturity: immature, semimature and mature; they observed that for both varieties of coffee, with an increase in the degree of maturity, the content of monocaffeoylquinic acids: 3-CQA increased, and the contents of 5-CQA and di-CQA decreased. Some differences were observed in fructose content at different degrees of maturity; immature and semimature Catuai samples had the highest content, while mature Catuai samples had the lowest. There was no difference between the degrees of maturity for Typica coffee.

Regarding sensory quality, some studies have determined that the best sensory attributes of the coffee beverage are obtained from ripe fruits [17]. However, green and

half-ripe fruits can have lower quality due to multiple defects, such as bitterness, graininess and sourness. Similarly, overripe and dried fruits exhibit defects ranging from fermentation to moldiness, strange flavors and unpleasant odors. Ref. [18] evaluated the volatile profiles of fruits at different stages of ripening, from partially ripe to overripe, and minimal differences were obtained; immature fruits were excluded from the analysis. These findings were similar to those for sensory quality, in which mature fruits and partially ripe, yellow–green fruits presented cups of similar quality to red-ripe fruits. Ref. [19] found that coffee seeds harvested in the ripe cherry state have a greater storage potential than those harvested in the half-ripe state. The variations in the physical, chemical and sensory properties of coffee fruit have been evaluated throughout the maturation scale, including green, half-ripe and overripe fruits, which are the states of maturation exhibiting differences with respect to the mature fruit. Changes in chemical compounds during ripening mainly include the insufficient development of bean markers that have a negative effect on quality [16]. Within the range of maturity in which coffee fruit can be classified as ripe, there are a wide range of characteristics; this study differentiated and evaluated three states of maturity with respect to color, weight, amount of mucilage, chemical composition and sensory profile, revealing differences that could be used to determine the optimal level of maturation at which the fruit retains its characteristics and provides the maximum sensory quality without entering the senescence stage.

2. Materials and Methods

2.1. Coffee Fruits

Coffee fruits of the species *C. arabica L.*, variety Castillo®, from Chinchiná, Caldas, Colombia, were collected in the main harvest of 2020 and 2021 in multiple passes [20], which ensured the maximum concentration of mature coffee. A total of 200 kg of fruit (working unit) was harvested, hydraulically classified to eliminate low-quality fruits and subsequently separated into three degrees of maturity. The first state was defined by a red–orange color (MS1); the second, a crimson color (MS2); and the third, a red wine color (MS3) (Figure 1). The agroclimatological characteristics of the area for 2020 were as follows: altitude 1381 m, minimum temperature 17.1 °C, maximum temperature 27.8 °C, average temperature 21.6 °C, relative humidity 80.3%, total precipitation 2470.9 mm, rainy days 222, and solar brightness 1440.9 h.

Figure 1. Maturity stages of coffee fruits.

The National Coffee Research Center (Cenicafé) uses the strategy of genetic diversity to obtain coffee varieties with durable resistance to coffee rust. Under this concept, the varieties developed by Cenicafé are compound varieties, which means that they are a mixture of different progenies that differ in their level of resistance but that share agronomic attributes such as high production and good physical and sensory quality of the bean. In 2005, it delivered the Castillo® variety adapted to specific coffee growing regions of Colombia. The Castillo® General variety stands out for its intermediate aspect, durable resistance to rust, tolerance to coffee cherry disease (CBD) and high sensory quality. They are composite varieties, made up of improved lines that are not necessarily similar in appearance, which means that different types of plant architecture and foliar colors (green and bronze) can be observed in the field, without this being a limiting factor in the development of their agronomic and productive potential.

The low-growing varieties grown in Colombia allow the use of higher planting densities compared to the densities used in tall varieties. The highest yields obtained with

low-growing varieties have been achieved with planting densities of 10,000 plants per hectare and for tall varieties with planting densities of 5000 plants per hectare. In general, for the intermediate varieties, planting densities between the above-mentioned range were used, sowing during the rainy season, after the main harvest of the area, which corresponds to the second half of the year, had been completed.

The soil characteristics of the experimental farm of Naranjal are as follows: pH 5.2, organic matter 13.3%, nitrogen 0.51%, phosphorus 0.51 ppm, taxonomic group Hapludands and parent material volcanic ashes. In coffee, the two most absorbed macronutrients at all stages of the crop are nitrogen and potassium; calcium, phosphorus, magnesium and sulfur are in second place. The nutritional requirements of coffee increase with age. At the beginning of the reproductive phase the plant increases its growth rate and with it the demand for nutrients increases. The productivity of coffee plantations depends to a great extent on adequate nutrition. In order to obtain high yields, it is necessary that the plants first form sufficient roots, stems, branches and leaves: organs that also demand nutrients, and without which it would be impossible to achieve this purpose. Through the supply of the following quantities of nutrients, the needs of the technified coffee plantations with high sowing densities and free exposure to the sun were met: 300 kg/ha/year of nitrogen-N, 260 kg/ha/year of potassium-K_2O and 50 kg/ha/year of phosphorus-P_2O_5, 50 kg/ha/year of magnesium-MgO and 50 kg/ha/year of sulfur-S. Fertilization is carried out at the beginning of the rainy season.

From each stage of maturity, a sample composed of 200 g of fruits was taken to quantitatively determine the color on the CIEL*a*b* scale with a Chroma Meter CR410 (Konica Minolta, Tokyo, Japan), the fresh and dry weight, and the composition of the exocarp (shell), mesocarp (mucilage) and endosperm (seed). A total of eight working units for each stage of maturity were wet processed as follows: within 6 h of collection, the exocarp was removed by means of a 2500 horizontal pulper with a circular sieve (JM Estrada, La Estrella, Colombia). Subsequently, the mucilage was removed by mechanical demucilagination, followed by mechanical drying at 40 °C until the bean reached a moisture content between 10 and 11.5%.

2.2. High-Performance Liquid Chromatography (HPLC)

The analytes of interest were hot extracted from ground green coffee using type I water as a solvent. For the separation and quantification of sugars, an Alliance 2690 HPLC system coupled to a 2414 refractive index detector was used with a Sugar Pack I column (Waters, 6.5 × 300 mm, 10 µm) at 85 °C and deionized water at a flow rate of 0.5 mL/min. Organic acids and alkaloids were determined using a Waters 600E HPLC system coupled to a diode array detector (DAD-996). A Hi-Plex H column (Agilent, 7.7 × 300 mm, 8 µm) was used for the separation of organic acids at 50 °C with a flow of 0.5 mL/min, deionized water (acidified with 0.01 M H_2SO_4) and detection at 210 nm. For the determination of alkaloids, a Symmetry C_{18} column (Waters, 4.6 mm × 250 mm, 100 Å, 5 µm) was used at 35 °C with a flow of 1.0 mL/min, a mixture of water, methanol and acetic acid (59:40:1 $v/v/v$) and detection at 273 nm. All compounds were identified by comparison of the retention times with standards and quantified by using a calibration curve with an external standard according to the compound of interest.

2.3. Gas Chromatography

The composition of free fatty acids present in the lipid fraction of coffee was determined by a gas chromatograph coupled to a mass selective detector (HP-6890—MSD 5893) according to the reference method AOAC 969.33. Free fatty acids were converted to their corresponding methyl esters with boron trifluoride in 20% methanol followed by two successive extractions with 1 mL of hexane. The separation was performed with an HP-5MS column (Agilent, 30 m, 0.25 mm, 0.25 µm) and HP-MS column (5% phenylmethylsiloxane 30 × 250 µm × 0.25 µm) with a temperature ramp of 20 °C/min from 90 °C to 260 °C and a constant helium flow of 1.2 mL/min. Structural confirmation of the fatty acids present in

the samples was performed based on their mass spectra, which were compared with those of the WILEY 275 and NIST98 databases.

2.4. Total Chlorogenic Acids, Total Lipids and Total Protein

The determination of total CGA was performed in a Beckman spectrophotometer (DU-650) at wavelengths of 265, 328 and 380 nm after extraction of CGA with aqueous methanol and purification with Carrez reagents. For quantification of the lipid fraction, Soxhlet extraction was performed according to the analysis method AOAC 945.16.

The determination of total protein was performed using the dry combustion method according to AOAC 990.03 (Dumas method) (AOAC 2005), which is based on the destruction of organic matter through combustion under controlled oxygen supply at very high temperatures, generating carbon and nitrogen gases, which are quantified by an IR detection cell and a thermal conductivity cell. The protein value was obtained by multiplying the percentage of nitrogen obtained from the elemental analysis by a factor of 6.25. The results are expressed as a percentage (g protein/100 g sample).

2.5. Physical and Sensory Quality

In the analysis of the physical quality of the dry parchment coffee, the moisture content, the percentage of waste, defective beans, black and sour beans, broken beans and percentage of healthy kernels were measured [21]. The sensory evaluation was performed following the Specialty Coffee Association (SCA) protocol with the participation of 5 certified Q-Grader tasters. This methodology included the preparation protocol (colorimetry of the roasted beans, roasting time, coffee proportion, grinding, temperature and water quality) and analysis temperature. The samples were roasted in a Probat BRZ2 roaster with an initial temperature of 200 °C, reaching an average minimum temperature of 156.8 °C in 4 min and ending with an average temperature of 208.7 °C in 10.4 min. All samples were in a range of roasting colorimetry between 55–65 Agtron/SCA and time between 8 and 12 min. The perceived quality of 10 coffee flavor attributes was recorded: fragrance/aroma, flavor, residual flavor, acidity, body, balance, uniformity, clean cup, sweetness, overall impression, defects, and total score.

2.6. Statistical analysis

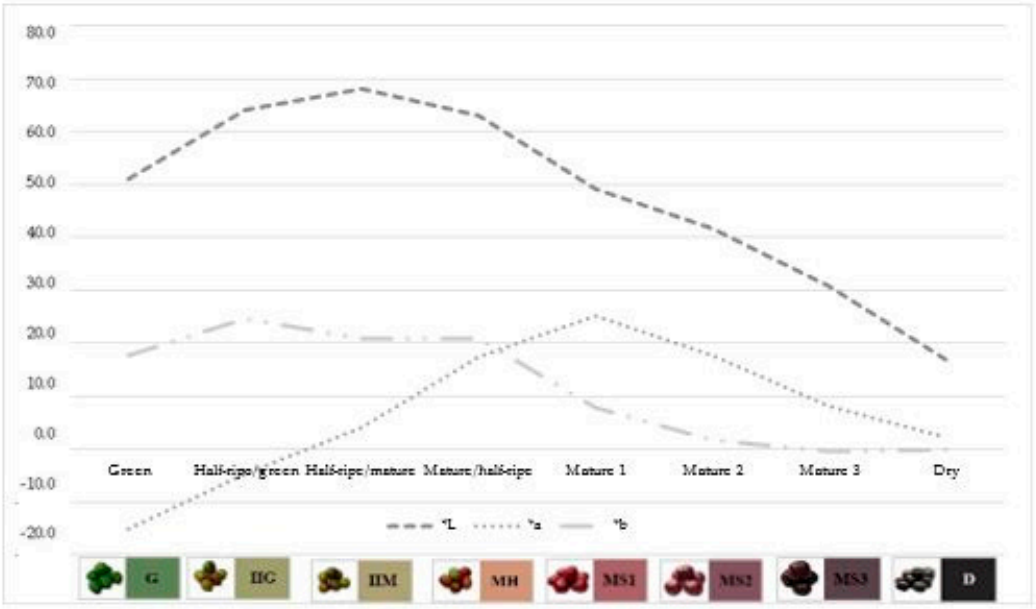
For each maturity stage, the response variables were analyzed with an analysis of variance corresponding to a completely randomized experimental design model at a significance level $\alpha = 0.05$. When the analysis of variance showed effect of treatments (p-value < 0.05), Duncan's multiple comparison test ($\alpha = 0.05$) was performed on the treatments to identify the effect of the maturity stage. A Pearson correlation coefficient matrix was developed using a heatmap to verify the relationships between chemical compounds and sensory attributes (SCA). This coefficient is a measure of linear dependence between two quantitative random variables: if the value is calculated as 1 if there is a perfect positive correlation and as -1 if there is a perfect negative correlation.

3. Results and Discussion

3.1. Colorimetry and Composition of Coffee Fruits

The chromatic coordinates of the exocarp, the weight of fresh and dried fruits, and the composition of the fruit expressed as % peel (exocarp), % mucilage (mesocarp) and % bean (seed) were evaluated over the entire maturity scale, starting with green (G), half-ripe/green (HG), half-ripe/mature (HM) and mature/half-ripe (MH); followed by the three maturity stages studied, MS1, MS2 and MS3; and ending with dry coffee (D). The coffee from the eight experimental units was separated into the states described above, and the fruits were measured with the color coordinates L*, a* and b*. The average weight of the fresh fruit was determined, and for the dry weight, the standard oven method at 105 °C was used according to ISO 6673 [22]. The composition of the fruit was determined by the weight of each of the components: peel, mucilage and bean.

The L* coordinate, which indicates luminosity, presents the maximum value in the HM state. The values for the HG and MH states are the same, and the values for the maturity stages MS1, MS2 and MS3 exhibit differences. As reported by [23], the luminosity L* does not show any significant effect in the prediction of the different physical or mechanical properties of the fruit. The minimum value was found in the D state. The chromatic coordinate a* has negative values in the green states and reaches a maximum value of 25.16 in MS1, and the values of the maturity stages MS1, MS2 and MS3 are different from each other. According to [23], the size of the fruit can be predicted from the color coordinate a*, and the fresh and dry mass of the coffee fruit can be predicted from a* and b*, with correlations of 92.1% and 78.5%, respectively. This correlation was confirmed by the maximum average values obtained for a* and the weight of the fresh fruit in MS1, where the maximum value of a* of 25.16 corresponds to the maximum fresh weight of the fruit of 2.07 g. The chromatic coordinate b* has a maximum value of 24.48 in the HG state due to its similarity to the color yellow, and the ripe fruits of MS1 are significantly different from those in the MS2 and MS3 states according to Duncan's test at 5% (Figure 2). The results for the chromatic coordinate b* differentiate the HG, HM and MH states from the three maturity stages evaluated, similar to the results found by [12], where the b* coordinate differentiated coffee fruits at 217 DAA (HG) from the mature fruits at 231 and 238 DAA (MS1 and MS3).



	L* Avg		a* Avg		b* Avg	
V	50.97	C	-15.18	G	17.71	C
PV	64.03	B	-4.84	F	24.48	A
PM	68.13	A	4.02	D	20.97	B
MP	63.05	B	17.42	B	20.95	B
MS1	49.01	D	25.16	A	7.74	D
MS2	41.58	E	17.55	B	1.71	E
MS3	30.84	F	8.09	C	-0.59	E
S	16.91	G	2.10	E	-0.07	E

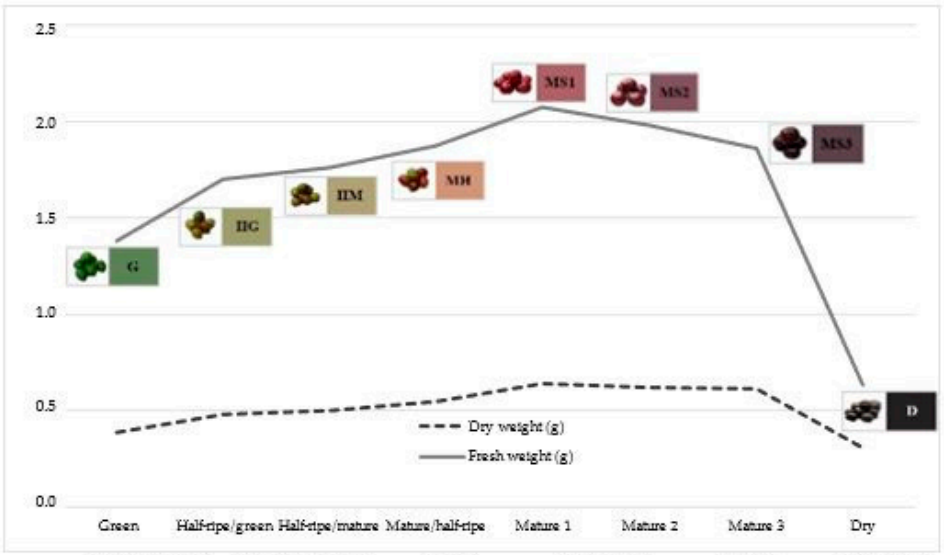
Figure 2. Colorimetry of coffee fruit by ripening stage. Different letters indicate a difference between means according to Duncan's test at 5%.

The average weight of fresh fruits varies between 0.63 and 2.07 for D and MS1, respectively. The weight for MS2 is not significantly different from those of MS1 and MS3, while

the weight of MS3 shows no difference with respect to HM. The weights of the fresh fruits in the green and dry states are different from those in the other states. The maximum dry weight values were found in the maturity stages MS1, MS2 and MS3, and there was no significant difference between them. As was observed for the fresh weight, the dry weights of the fruits of the green and dry states are different from those of the other states. The fresh weight of the MS1 maturity stage is 10.14% higher than that of the MS3 stage, while the dry weights of the three stages are statistically equal. This loss of fresh weight between maturity stages 1 and 3 can be associated with the dehydration processes that the fruit undergoes in the last phase of ripening [1,6].

After the dry weight reached the maximum values in the three stages of maturity evaluated, it decreased to the minimum value of 0.3 g in the dry state, defined for its deteriorated appearance associated with the absence of mucilage and lower water content. Once maturity is reached, the seed loses weight, which can be explained by the interruption of the translocation of photoassimilates from the fruit to the seed, as well as by the consumption of substrate necessary for respiration during the stages of full maturity [5].

The mucilage contents in the maturity stages MS1 and MS2 are the same but are different from that of MS3; the maximum is found for MS1 with a value of 15.44, and the value decreases as the state of maturity increases until reaching 10.07. This mucilage content of the MS3 stage is equal to that in the HG and HM stages. The bean content presents a behavior similar to that of mucilage; the percentages in the stages of maturity MS1 and MS2 are equal but are different from that in MS3, and the value for MS3 is equal to that of HM (Figure 3). The processing of fruits of the MS3 stage, which involves the use of a demucilator, implies greater defects; since the mucilage content is lower, there is a greater risk of generating physical defects such as cracked, broken or threshed beans.



	Dry weight (g) Avg		Fresh weight (g) Avg		% Peel Avg		% Mucilage Avg		% Bean Avg		Conversion Avg	
V	0.38	D	1.38	D	Nd		Nd		Nd		Nd	
PV	0.48	C	1.70	C	41.38	D	8.71	D	47.36	A	4.18	C
PM	0.50	CB	1.76	C	42.60	DC	11.12	C	44.22	B	4.48	B
MP	0.54	B	1.87	BC	42.94	DC	13.33	B	41.46	C	4.63	B
MS1	0.64	A	2.07	A	43.98	BC	15.44	A	38.92	D	4.86	A
MS2	0.62	A	1.98	BA	45.84	BA	14.10	BA	38.14	D	4.94	A
MS3	0.61	A	1.86	BC	46.84	A	10.07	DC	40.91	C	4.56	B
S	0.30	E	0.63	E	Nd		Nd		Nd		Nd	

Figure 3. Fresh and dry weight, and composition of coffee fruit by ripening stage. Different letters indicate a difference between means according to Duncan's test at 5%.

Conversion refers to the amount of coffee fruits (kg) required to obtain 1 kg of dry parchment coffee; normally, for wet-processed coffees, this conversion corresponds to 5 kg

of mature coffee. As in the case of mucilage and bean, the conversion in the maturity stages MS1 and MS2 is the same but different from that in MS3, and the conversion in MS3 is the same as that in the HG and HM stages. The lowest value among the maturity stages MS1, MS2 and MS3 is observed for MS3 with a value of 4.56. The conversion of fruits in the MS3 stage coincides with the value of 4.51 for a mixture of mature and overripe fruits reported by [20] reported a value. This result implies that to obtain the same amount of dry parchment coffee, if a mass composed entirely of fruits in the state of maturity MS3 is used, 8.8% less of the total mass is processed.

3.2. Physical Quality

The analysis of the physical quality of the dry parchment coffee included the measurement of the following variables: moisture content, percent loss, defective beans, black and sour beans, broken beans and percentage of healthy kernels. The percent loss refers to the amount of parchment (endocarp) that the dry bean has. Regarding the physical quality variables, there were no significant differences between the stages of maturity evaluated. In the case of the content of broken beans, it is important to clarify that in the multiple-pass harvesting method, to optimize the concentration of mature fruits, levels of borer infestation less than 2% are required before starting the harvest process to preserve physical quality.

The average moisture content of the samples was 11.3%, which implied that the average water activity was 0.6216. The percent loss had an average value of 18.54, and the minimum and maximum values of 16.5 and 21.1 were found in the maturity stages MS2 and MS3, respectively. The maximum content of defective beans was presented by MS3 with a value of 4.06; for black and sour beans, the maximum value was found in MS2 with a value of 0.81. Ref. [24] evidenced a negative correlation between the number of black and insect-attacked beans and the quality of the coffee beverage. Physical quality can be affected by postharvest process. Ref. [25] reports color differences between coffee beans from freshly processed and soaked fruits, attributing it to the effect of leaching of color pigments from the outer layer of the coffee bean. The average content of broken beans was 3.27%, and the maximum value was found in MS2 with a value of 10.79%. The percentage of healthy kernels refers to the number of healthy kernels without any defects present in a given amount of coffee. The average value for washed coffee is estimated at 74%, and higher values indicate a better physical quality. The percentages of healthy kernels were 75.71, 76.07 and 74.75 for the maturity stages MS1, MS2 and MS3, respectively. The maximum value of healthy kernels was reported for MS1 with a value of 80.78%.

3.3. Organic Acids

The contents of the different organic acids did not show significant differences between the stages of maturity evaluated. MS1 presented the maximum values for lactic and succinic acids, MS2 presented the maximum values for acetic, citric, malic and quinic acids, and MS3 presented the maximum value for oxalic acid (Figure 4). The major acids found followed the order citric acid, quinic acid, malic acid and acetic acid, the first three coinciding with those reported by [26]. These authors found different accumulation patterns of carboxylic acids in the pericarp and in the seeds, and the most significant difference was found in the accumulation of citric acid, which is one of the main carboxylic acids in the seeds. Among the evaluated acids, this study obtained an average and maximum value of 9.28 g/kg. During fruit ripening, quinic acid, which is a precursor of all CGAs, increases slightly in the pericarp and endosperm, suggesting that it is transported through the perisperm to the endosperm [6]. However, in this study, there is no evidence of an increase in this acid in the coffee seed in advanced stages of fruit maturity.

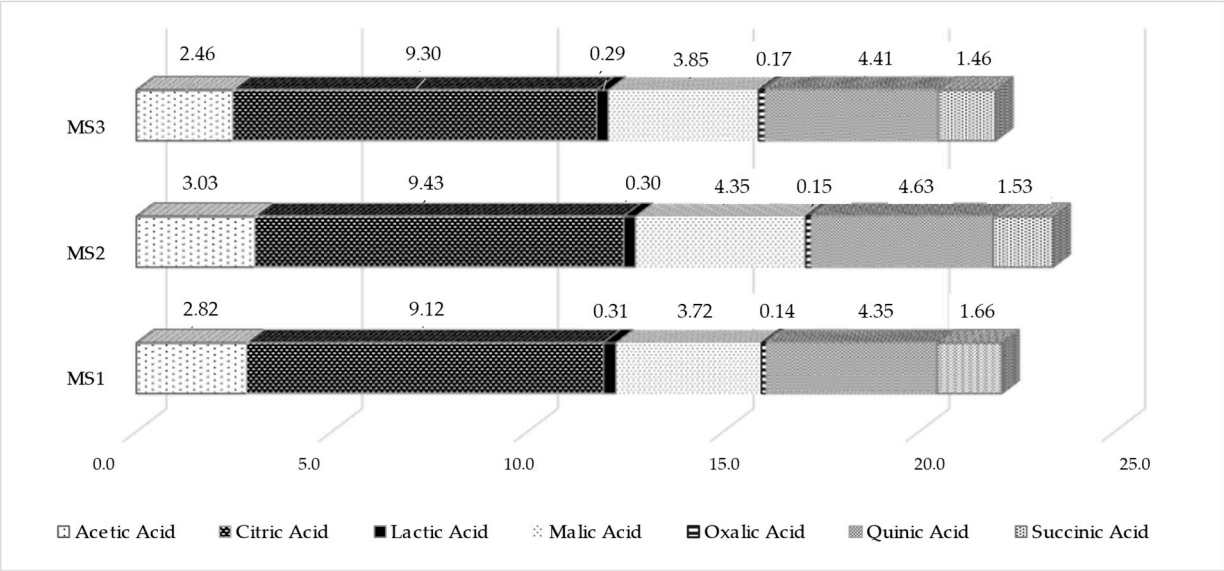


Figure 4. Average content (g/kg) of organic acids for three stages of maturity of coffee fruit.

3.4. Lipids and Fatty Acids

Lipids in coffee have an important impact on the final quality of the product due to the formation of oxidation products over time [27]. Among the fatty acids present in coffee, linoleic acid is the predominant fatty acid, followed by palmitic acid. The coffee kernel waxes, together with the hydroxytryptamide esters of various fatty acids (arachidic, behenic and lignoceric), originate in the epicarp of the fruit [28]. Regarding the average values of fatty acids, palmitic acid was the predominant fatty acid, followed by linoleic, oleic, stearic and arachidic acids. The analysis of variance did not show a significant effect of maturity stage on the lipid fraction or on the composition of fatty acids.

The maximum average lipid fraction was found in the state of maturity MS2 with a value of 10.45. Regarding the composition of fatty acids, MS1 presented the maximum values for palmitic and oleic fatty acids, and MS3 presented the maximum values for stearic and arachidic fatty acids. Linoleic fatty acid had an equal maximum value for maturity stages MS1 and MS3 (see Table 1).

3.5. Total Protein and Alkaloids

The analysis of variance did not show a significant effect of maturity stage on total protein and alkaloids. The total protein presented the minimum value in MS3 and the maximum in MS2 (Table 1). Ref. [4] reported that in mature beans of *C. arabica* var. Caturra, the protein content represents 10% of the dry weight. MS2 presented the maximum value for caffeine and MS3 presented the maximum value for trigonelline; theobromine exhibited equal values for the three stages of maturity. During ripening, caffeine accumulates in the endosperm and decreases in the pericarp [6]. The equal values obtained for the three stages of maturity agree with the study of [4], in which the caffeine content in seeds was consistent with the dry weight curve and remained constant during maturation, close to 1% on a dry basis. Unlike caffeine, other compounds are synthesized in all parts of the coffee tree, as well as in the pericarp and beans, generating transport from the chlorophyll tissues to the endosperm. This behavior is shown by trigonelline, which is synthesized in the pericarp and transferred to the seeds [29]. This process is evidenced in this study, where the content of trigonelline increases as the maturity stage advances; the MS1 state has an average content of 0.85, MS2 has an average content of 0.86 and MS3 has an average content of 0. 87.

Table 1. * Average contents of lipids, free fatty acids, protein, alkaloids and total chlorogenic acids for three stages of maturity of the coffee fruit.

	MS1				MS2				MS3			
	PROM	**STD**	**MIN**	**MAX**	**PROM**	**STD**	**MIN**	**MAX**	**PROM**	**STD**	**MIN**	**MAX**
Lipids (%)	10.28	0.54	9.50	11.03	10.45	0.77	9.45	11.80	10.38	0.60	9.37	10.93
Palmitic acid (%)	41.23	1.51	39.07	43.52	41.18	1.26	38.85	42.76	41.15	1.84	39.16	45.27
Linoleic acid (%)	34.03	1.15	32.26	35.82	33.91	1.78	30.24	35.74	34.03	1.72	31.47	36.72
Oleic acid (%)	10.66	0.83	9.44	11.80	10.47	0.94	8.75	11.96	10.45	0.93	9.46	12.00
Stearic acid (%)	9.42	0.39	8.74	9.87	9.60	0.49	8.68	10.22	9.62	0.55	9.16	10.46
Arachidic acid (%)	4.66	0.48	4.08	5.41	4.71	0.72	3.84	5.68	4.77	0.69	3.77	5.85
Protein (%)	13.93	1.01	12.69	15.38	14.52	1.48	12.81	17.44	13.92	1.04	12.69	15.19
Caffeine (%)	1.09	0.11	0.93	1.27	1.11	0.16	0.91	1.46	1.10	0.06	1.04	1.23
Theobromine (%)	0.03	0.01	0.02	0.08	0.03	0.01	0.02	0.08	0.03	0.01	0.01	0.08
Trigonellin (%)	0.85	0.08	0.76	0.98	0.86	0.09	0.76	1.02	0.87	0.11	0.66	0.99
Total chlorogenic acids (%)	4.18	0.34	3.57	4.70	4.29	0.45	3.83	5.10	4.09	0.46	3.47	4.88

* For the compounds in Table 1, the analysis of variance showed no maturity stage effect.

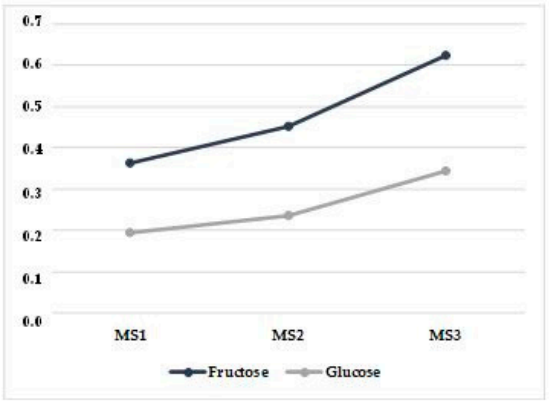
3.6. Total Chlorogenic Acids

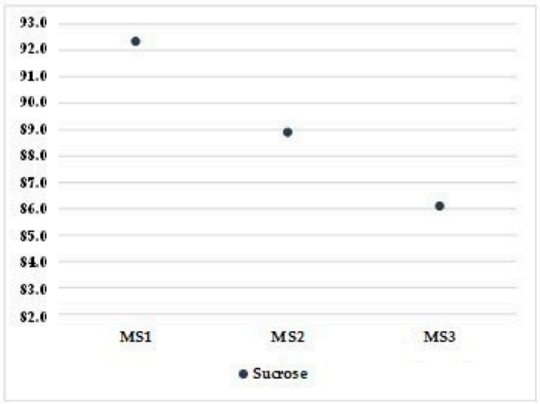
The minimum and maximum average values of total CGA were found in maturity stages MS3 and MS2, respectively (Table 1). The analysis of variance did not show a significant effect of maturity stage on total CGA. In a study conducted by [30] for arabica coffee, the highest average total CGA content was found in the green state. The average CGA content for the three stages of maturity is 4.19, which is different from that reported by [30] but similar to that found by [16]: the first authors report a value of 6.23 for mature arabica coffee, and the second reports values of 4.1 and 4.6 for mature fruits of the Catuai and Tipica varieties, respectively. Similar to this study, they did not find significant differences between the total CGA contents for the different stages of fruit ripening. Compared to mature beans, immature beans are more sensitive to the oxidation of CGAs [31].

3.7. Sugars

In coffee fruits, sucrose, glucose and fructose are the main free sugars. During fruit ripening, the sugar content increases in the pericarp and in the seeds; sucrose can correspond to 73% of the total sugars in the seeds [26]. In this study, the analysis of variance showed a significant effect of maturity stage on fructose and glucose, but not sucrose. The fructose content is highest in the MS3 state (0.62) and is different from that of the MS1 (0.36) and MS2 (0.45) states. Glucose has a maximum value in the MS3 stage (0.49) and is different for the three stages of maturity (Figure 5), according to Duncan's test at 5%. The higher values of fructose and glucose with increasing state of maturity can be related to the hydrolysis of sucrose, whose content decreases from 92.34 to 86.09 in the MS1 to MS3 stages. This behavior is similar to that observed in dry (natural)-processed coffees; the hydrolysis of sucrose is favored by the presence of water in the fruit, increasing the contents of fructose and glucose. In the case of natural coffee, this behavior may be a response to longer processing times of fruit in the drying stage, where the removal of water is more gradual than in wet-processed coffees. In the advanced stages of maturity, this trend can be associated with longer periods of permanence of the fruit on the tree. Although sucrose decreases, it is the main sugar of the seed according to [26]. Ref. [32] reported that sucrose levels in *Coffea racemosa* are relatively high and stable during the development of the endosperm, and this differs significantly from the situation observed in other coffee species, in which sucrose contents were low in the young endosperm and increased just before harvest [6], suggesting that sucrose is transported directly to the endosperm from chlorophyll tissues. As the maturation phase progresses, the accumulation of proteins, sucrose and complex polysaccharides represent the main reserves of the seed [4]. After reaching maturity, no further accumulation of sucrose is generated in the fruit, but the contents of glucose and fructose increase; nevertheless, these changes do not modify the total sugar content. The effect of this change is not evident in sensory quality since, as reported by [33], the perceptible sweetness in coffee is a consequence of the presence of

aromas and flavors associated with sweetness. Although a change in the concentration of these sugars occurs, the individual effect is not enough to generate changes in the flavor attribute that will ultimately be reflected in the final score of the drink.





	Fructose (g/kg)				Glucose (g/kg)				Sucrose (g/kg)			
	AVG	STD	MIN	MAX	AVG	STD	MIN	MAX	AVG	STD	MIN	MAX
MS1	0.36 B	0.08	0.28	0.48	0.19 C	0.02	0.15	0.23	92.34	2.32	88.57	95.34
MS2	0.45 B	0.13	0.25	0.65	0.23 B	0.03	0.21	0.29	88.92	4.61	79.15	93.09
MS3	0.62 A	0.09	0.49	0.73	0.34 A	0.03	0.28	0.39	86.09	4.49	80.32	91.76

Figure 5. Sugar content by state of maturity. Different letters indicate a difference between averages according to Duncan's test at 5%.

3.8. Sensory Quality

The total SCA score is calculated from the sum of ten sensory attributes of coffee. According to the scale, coffees with scores equal to or greater than 80 points are considered special for their sensory quality. The analysis of variance did not show a significant effect of the state of maturity on the total ACS score or on the sensory attributes. For the fragrance/aroma attribute, MS1 obtained the maximum average value, and the maximum average values of the other attributes, flavor, residual flavor, acidity, body, balance, taste score and total SCA score were obtained in MS2. The average total SCA scores were 82.73, 82.79 and 82.43 for MS1, MS2 and MS3, respectively, and the corresponding maximum scores were 83.75, 84.08 and 83.5. This result coincides with [18], who evaluated the volatile profiles at different stages of maturation, excluding samples of immature fruits; the profiles showed minimal differences. This finding is similar to the results reported by the same authors on the sensory quality and [30], in which mature, partially ripe and yellow–green fruits presented cups of similar quality to red-ripe fruits. The three degrees of maturity evaluated did not show differences in the sensory attributes or in the final sensory quality expressed as a total SCA score, which indicates that the stage of maturity MS3 should not be considered overmature, since this score implies negative considerations for quality. MS3 can be considered an advanced stage of maturity in coffee fruit.

With respect to the sensory descriptors of flavor, the tasters identified a total of 125, which were classified into the following groups: caramel-sweet, chocolate, citrus, spices, floral, fruit and nuts. Of the total descriptors, 37.60% correspond to the caramel-sweet group, 24.80% to chocolate, 16.80% to nuts, which include walnut, hazelnut and almond flavors, 10.40% to fruit, and the remaining 10.40% to the citrus, spice and floral groups. The highest frequency of descriptors of the caramel-sweet group was found for MS2 with a value of 40.48%. MS2 also presented the lowest frequency of descriptors of the chocolate group (19.05%), and the maximum frequency for this group was associated with MS3, with a value of 30.30%. The fruit group had a similar behavior to the chocolate group: the frequency was initially 8.08% in MS1, increased to the maximum in MS2 with 19.05%, and ended with a value of 3.03% in MS3.

To verify the relationships between chemical compounds and SCA sensory attributes, a Pearson correlation coefficient matrix was constructed using a heatmap (Figure 6). This coefficient is a measure of linear dependence between two quantitative variables: if the value is 1, there is a perfect positive correlation, and a value of −1 denotes a perfect negative correlation. The chemical compounds with maximum positive correlations with the sensory attributes are citric acid (0.313), oleic fatty acid (0.591), theobromine (0.205), trigonelline (0.333) and protein (0.266) with the fragrance/aroma; lactic acid (0.193) and stearic fatty acid (0.123) with flavor; palmitic fatty acid (0.299) and sucrose (0.196) with residual flavor; malic acid (0.168) and quinic acid (0.111) with acidity; acetic acid (0.287), succinic acid (0.353) and arachidic fatty acid (0.156) with the body of the beverage; and the balance is related to oxalic acid (0.297).

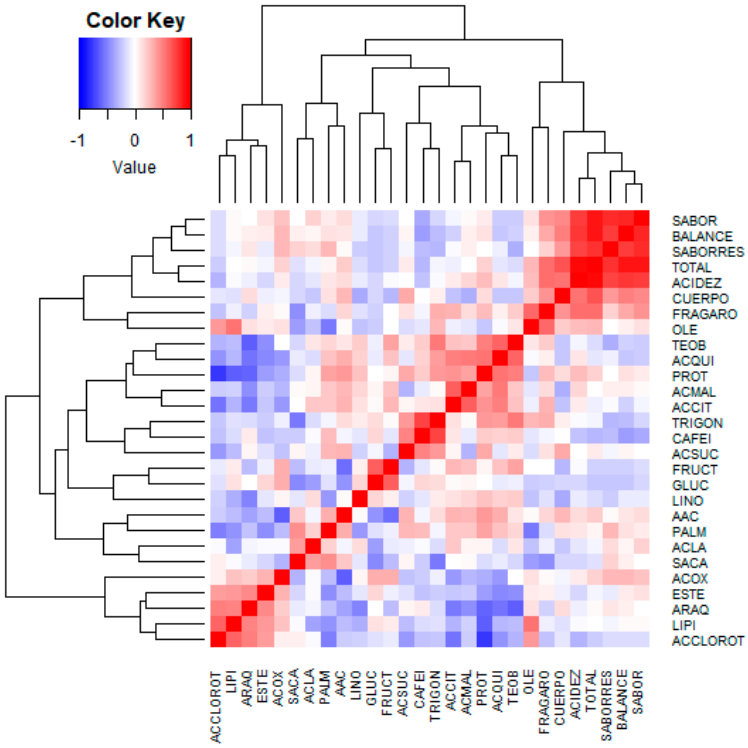


Figure 6. Heatmap of green coffee kernel chemical compounds and sensory attributes.

Relationships were also established between the sensory attributes and the total score of the samples. The attributes with the highest correlation values with the total SCA score are acidity, flavor and balance, with values of 0.922, 0.894 and 0.881, respectively. These same attributes are correlated with each other, with a correlation between acidity and balance of 0.774 and between flavor and residual flavor of 0.807. Relative to the total score, correlation value of residual flavor is 0.792, that of fragrance/aroma is 0.574 and that of body is 0.674. The compounds that show a positive correlation with the total score are acetic acid, oxalic acid, oleic fatty acid and protein, with correlation values of 0.207, 0.195, 0.299 and 0.187, respectively. The above results suggest that a single compound does not have an overall effect on the sensory attributes and in turn on the total rating; in contrast, all chemical compounds contribute to and have an integral effect on the characteristics that make up the sensory quality of coffee.

Among the chemical compounds, citric acid is positively related to malic acid (0.636), and these two compounds are among the major organic acids quantified in this study for green coffee beans, with average contents of 9.28 and 3.97 g/kg, respectively. Lipids show correlation values of 0.551, 0.478 and 0.410 with oleic, arachidic and stearic free fatty acids. Stearic acid has a correlation value of 0.476 with arachidic acid. In the group of alkaloids,

trigonelline is positively related to theobromine and caffeine, with values of 0.540 and 0.723, respectively. Fructose and glucose have a correlation value of 0.630.

4. Conclusions

The sensory quality of coffee is influenced by multiple factors that begin in the tree where the chemical precursors of aromas and flavors are deposited and formed in the seeds as the fruits develop. Although the sensory complexity is further developed throughout the different processing steps, it is important to know the particularities of the raw material that will be processed; for this reason, it is necessary to determine the physical, chemical and sensory characteristics associated with different degrees maturity of coffee fruits. Coffee fruits that have not fully developed can have overall negative effects on quality, but within the range in which the coffee fruit can be classified as ripe there are a wide range of differences. In this study, various characteristics were evaluated to define the maximum level of maturation, and the results indicated that in the three maturity stages evaluated, the fruit preserves its characteristics and allows the maximum quality without entering the senescence stage. The degrees of maturity evaluated did not show significant differences in the chemical compounds evaluated, with the exception of fructose and glucose, which did not generate changes in the sensory attributes or in the final sensory quality. This indicates that advanced stages of maturity where the fruit has not lost its turgor should not be considered overripe, since this definition implies negative effects on quality. Confirming that different stages of maturity do not have differences and do not generate a negative effect on the characteristics of the bean will allow coffee growers to optimize the harvesting process by collecting a higher concentration of fruits that are ripe and in advanced stages of maturity, enabling the processing of a smaller quantity of fresh coffee because of the lower mucilage content. In future work, it will be important to determine the effect of coffee fruits at different degrees of maturity in different wet processing stages that involve contact between the bean and the exocarp and mesocarp; the resulting changes in the final chemical composition of the coffee bean and in sensory quality; and the mechanisms driving these changes. The results of this study contribute to understanding the most suitable processes for achieving the maximum expression of the attributes of coffee and a consistent final quality.

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References

1. Ocampo, D.M.; Riaño, N.M.; López, J.C.; López, Y. Intercambio de dióxido de carbono y cambios bioquímicos en el pericarpio durante el desarrollo del fruto del cafeto. *Rev. Cenicafé* **2010**, *61*, 327–343. Available online: http://hdl.handle.net/10778/507 (accessed on 2 September 2022).
2. Esquivel, P.; Jiménez, V.M. Functional properties of coffee and coffee by-products. *Food Res. Int.* **2012**, *46*, 488–495. [CrossRef]
3. Salazar, M.R.; Riaño-Herrera, N.M.; Arcila Pulgarín, J.; Ponce, C.A. Estudio morfológico anatómico y ultraestructural del fruto de café *Coffea arabica* L. *Rev. Cenicafé* **1994**, *45*, 93–105. Available online: http://hdl.handle.net/10778/795 (accessed on 2 September 2022).

4. De Castro, R.D.; Marraccini, P. Cytology, biochemistry and molecular changes during coffee fruit development. *Braz. J. Plant Physiol.* **2006**, *18*, 175–199. [CrossRef]
5. Eira, M.T.S.; Silva EAA da De Castro, R.D.; Dussert, S.; Walters, C.; Bewley, J.D.; Hilhorst, H.W.M. Coffee seed physiology. *Braz. J. Plant Physiol.* **2006**, *18*, 149–163. [CrossRef]
6. Marraccini, P.; Allard, C.; Andre, M.; Courjault, C.; Gaborit, C.; Lacoste, N.; Meunier, A.; Michaux, S.; Petit, V.; Priyono, P.; et al. Update on coffee biochemical compounds, protein and gene expression during bean maturation and in other tissues. In Proceedings of the 19th International Scientific Colloquium on Coffee, Trieste, Italy, 14–18 May 2001. Available online: https://www.asic-cafe.org/conference/19th-international-scientific-colloquium-coffee (accessed on 12 July 2022).
7. Puerta, G.I.; Rios-Arias, S. Composición química del mucílago de café, según el tiempo de fermemtación y refrigeración. *Rev. Cenicafé* **2011**, *62*, 23–40. Available online: http://hdl.handle.net/10778/478 (accessed on 17 May 2022).
8. Amorim, A.C.L.; Hovell, A.M.C.; Pinto, A.C.; Eberlin, M.N.; Arruda, N.P.; Pereira, E.J.; Bizzo, H.R.; Catharino, R.R.; Filho, Z.B.; Rezende, C.M. Green and roasted arabica coffees differentiated by ripeness, process and cup quality via electrospray ionization mass spectrometry fingerprinting. *J. Braz. Chem. Soc.* **2009**, *20*, 313–321. [CrossRef]
9. Wallis-García, J.A.; Montoya, E.C.; Vélez-Zape, J.C.; Oliveros, C.E. Calidad y eficacia de dos métodos no selectivos de recolección manual de café *Coffea arabica*. *Rev. Cenicafé* **2004**, *55*, 45–51. Available online: http://hdl.handle.net/10778/113 (accessed on 24 June 2022).
10. Marín, S.M.; Arcila-Pulgarín, J.; Montoya-Restrepo, E.C.; Oliveros-Tascón, C.E. Cambios físicos y químicos durante la maduración del fruto de café (*Coffea arabica* L. var. Colombia). *Rev. Cenicafé* **2003**, *54*, 208–225. Available online: http://hdl.handle.net/10778/265 (accessed on 18 May 2022).
11. Martinez, V.M.; Aristizábal, I.D.; Moreno, E.L. Evaluation of the composition effect of harvested coffee in the organoleptic properties of coffee drink. *Vitae* **2017**, *24*, 47–58. [CrossRef]
12. Carvajal, J.J.; Aristizábal, I.D.; Oliveros, C.E.; Mejía, J.W. Coffee Fruit (*Coffea arabica* L.) Colorimetry During its Development and Maturation. *Rev. Fac. Nac. Agron. Medellín* **2011**, *64*, 6229–6240. Available online: https://revistas.unal.edu.co/index.php/refame/article/view/29414 (accessed on 13 July 2022).
13. Prasanna, V.; Prabha, T.N.; Tharanathan, R.N. Fruit Ripening Phenomena–An Overview. *Crit. Rev. Food Sci. Nutr.* **2007**, *47*, 1–19. [CrossRef] [PubMed]
14. Rogers, W.J.; Michaux, S.; Bastin, M.; Bucheli, P. 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 Sci.* **1999**, *149*, 115–123. [CrossRef]
15. Koshiro, Y.; Jackson, M.C.; Katahira, R.; Wang, M.-L.; Nagai, C.; Ashihara, H. Biosynthesis of Chlorogenic Acids in Growing and Ripening Fruits of *Coffea arabica* and *Coffea canephora* Plants. *Z. Nat. C* **2007**, *62*, 731–742. [CrossRef] [PubMed]
16. Smrke, S.; Kroslakova, I.; Gloess, A.N.; Yeretzian, C. Differentiation of degrees of ripeness of Catuai and Tipica green coffee by chromatographical and statistical techniques. *Food Chem.* **2015**, *174*, 637–642. [CrossRef]
17. Barboza, C.A.; Amaya, F.L. Quality analysis of coffee var. Caturra beans and beverage as a function of berry ripeness and vean fermentation time. *Agron. Trop.* **1996**, *46*, 289–311.
18. Velásquez, S.; Peña, N.; Bohórquez, J.C.; Gutierrez, N.; Sacks, G.L. Volatile and sensory characterization of roast coffees—Effects of cherry maturity. *Food Chem.* **2019**, *274*, 137–145. [CrossRef]
19. Veiga, A.D.; Guimarães, R.M.; Rosa, S.D.V.F.; Von Pinho, É.V.; Silva, L.H.; Veiga, A.D. Storability of coffee seeds harvestedat different maturation stages and submitted to different drying methods. *Rev. Bras. Sementes* **2007**, *29*, 83–91. [CrossRef]
20. Sanz, J.R.; Oliveros, C.E.; Duque, H.; Mejía, C.G.; Benavides, P.; Rivera, R.D. Retención de pases: Una opción para mejorar la productividad de la mano de obra en la cosecha de café. *Av. Técnicos Cenicafé* **2018**, *488*, 1–8. Available online: http://hdl.handle.net/10778/4218 (accessed on 3 August 2022). [CrossRef]
21. *NTC 2324*; Café verde. Examen Olfativo y Visual y Determinación de Materia Extraña y Defectos. Instituto Colombiano de Normas Técnicas y Certificación: Bogotá, Colombia, 2021. Available online: https://tienda.icontec.org/gp-cafe-verde-examen-olfativo-y-visual-y-determinacion-de-materia-extrana-y-defectos-ntc2324-2021.htm (accessed on 2 February 2022).
22. *ISO 6673:2003*; Green Coffee—Determination of Loss in Mass at 105 Degrees C. International Organization for Standardization [ISO]: Geneva, Switzerland, 2003. Available online: https://www.iso.org/obp/ui/#iso:std:iso:6673:ed-2:v1:en (accessed on 2 February 2022).
23. Carvajal, J.J.; Aristizábal, I.D.; Oliveros, C.E. Physical and mechanical properties evaluation of coffee fruit (Coffea Arabica L. var. Colombia) during its development and maturation. *DYNA* **2012**, *79*, 116–124. Available online: https://revistas.unal.edu.co/index.php/dyna/article/view/25965 (accessed on 28 July 2022).
24. Vargas, E.A.; Silva, F.B.; Santos, E.A.; Souza, S.M.C.; Souza, S.E.; Correa, T.B.S.; Franca, R.C.A.; Amorim, S.S.; Pfenning, L.H.; Batista, L.R.; et al. Influence of Coffee Processing and Defects on the Incidence and Occurrence of Ochratoxin A. *Int. Conf. Coffee Sci.* **2005**, 410–417, Association for Science and Information on Coffee, 2005. 1 CD-ROM.
25. Ikumi, P.W.; Koskei, R.K.; Njoroge, D.M.; Kathurima, C.W. Effect of Soaking Coffee (*Coffea arabica*) Cherries on Biochemical Composition and Cup Quality of Coffee Brew. *IOSR J. Environ. Sci. Toxicol. Food Technol.* **2017**, *11*, 14–18. [CrossRef]
26. Koshiro, Y.; Jackson, M.C.; Nagai, C.; Ashihara, H. Changes in the content of sugars and organic acids during ripening of *Coffea arabica* and *Coffea canephora* fruits. *Eur. Chem. Bull.* **2022**, *4*, 378–383. Available online: https://www.eurchembull.com/?mno=66742 (accessed on 2 February 2022).

27. Ortolá, M.D.; Gutiérrez, C.L.; Chiralt, A.; Fito, P. Kinetic study of lipid oxidation in roasted coffee. *Food Sci. Technol. Int.* **1998**, *4*, 67–73. [CrossRef]
28. Belitz, H.D.; Grosch, W.; Schieberle, P. Coffee, Tea, Cocoa. In *Food Chemistry*; Springer: Berlin/Heidelberg, Germany, 2009; pp. 938–970. [CrossRef]
29. Nagai, C.; Koshiro, Y.; Ashihara, H.; Wang, M.-L.; Zheng, X.-Q. Changes in content and biosynthetic activity of caffeine and trigonelline during growth and ripening of *Coffea arabica* and *Coffea canephora* fruits. *Plant Sci.* **2006**, *171*, 242–250. [CrossRef]
30. Marín, C.; Puerta, G.I. Contenido de ácidos clorogénicos en granos de *Coffea arabica* L. y *C. canephora*, según el desarrollo del fruto. *Rev. Cenicafé* **2008**, *59*, 7–28. Available online: http://hdl.handle.net/10778/60 (accessed on 2 February 2022).
31. Montavon, P.; Duruz, E.; Rumo, G.; Pratz, G. Evolution of Green Coffee Protein Profiles with Maturation and Relationship to Coffee Cup Quality. *J. Agric. Food Chem.* **2003**, *51*, 2328–2334. [CrossRef]
32. Geromel, C.; Ferreira, L.P.; Bottcher, A.; Pot, D.; Pereira, L.F.P.; Leroy, T.; Vieira, L.G.E.; Mazzafera, P.; Marraccini, P. Sucrose metabolism during fruit development in Coffea racemosa. *Ann. Appl. Biol.* **2008**, *152*, 179–187. [CrossRef]
33. Batali, M.E.; Frost, S.C.; Lebrilla, C.B.; Ristenpart, W.D.; Guinard, J. Sensory and monosaccharide analysis of drip brew coffee fractions *versus* brewing time. *J. Sci. Food Agric.* **2020**, *100*, 2953–2962. [CrossRef]