

# Physical and sensorial quality of arabica coffee cultivars submitted to two types of post-harvesting processing

Ricardo Dias Alixandre<sup>1</sup> , Fabiano Tristão Alixandre<sup>2</sup> , Paula Aparecida Muniz de Lima<sup>1</sup> , Maurício José Fornazier<sup>3</sup> , Cesar Abel Krohling<sup>4</sup> , José Francisco Teixeira do Amaral<sup>5</sup> , Rogério Carvalho Guarçoni<sup>3</sup> , Rodrigo da Silva Dias<sup>6</sup> , Cassio de Faria Venturini<sup>7</sup> , Higor Alixandre Macette<sup>1</sup> , Cecília Uliana Zandonadi<sup>8</sup> , David Brunelli Viçosi<sup>8</sup> 

<sup>1</sup>Universidade Federal do Espírito Santo/UFES, Programa de Pós-graduação em Agronomia, Campus Alegre, Alegre, ES, Brasil

<sup>2</sup>Universidade Federal do Espírito Santo/UFES, Programa de Pós-Graduação em Genética e Melhoramento, Campus Alegre, Alegre, ES, Brasil

<sup>3</sup>Instituto Capixaba de Pesquisa, Assistência Técnica e Extensão Rural/INCAPER, Venda Nova do Imigrante, ES, Brasil

<sup>4</sup>Instituto Capixaba de Pesquisa, Assistência Técnica e Extensão Rural/INCAPER, Marechal Floriano, ES, Brasil

<sup>5</sup>Universidade Federal do Espírito Santo/UFES, Departamento de Agronomia, Alegre, ES, Brasil

<sup>6</sup>Inove Consultoria, Venda Nova do Imigrante, ES, Brasil

<sup>7</sup>Instituto Federal de Educação, Ciência e Tecnologia do Espírito Santo/IFES, Santa Teresa, ES, Brasil

<sup>8</sup>Fundação de Amparo à Pesquisa e Inovação do Espírito Santo, Venda Nova do Imigrante, ES, Brasil

Contact authors: ricardoalixandre@gmail.com; fabianotristao@incaper.es.gov.br; aluap-lima@hotmail.com; mauriciofornazier@gmail.com; cakrohling@gmail.com; jftamaral@yahoo.com.br; rogerio.guarconi@gmail.com; rodrigodasilvadias@yahoo.com.br; cassiofventurini@gmail.com; higorlimacette@gmail.com; ceciliauli@hotmail.com; davidvicosi@hotmail.com

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## ABSTRACT

The growing demand for better quality coffees has driven changes in the coffee production chain, mainly through the adoption of new technologies and management. Thus, this work was carried out aiming to evaluate the physical and sensorial quality of Arabica coffee cultivars, submitted to natural and pulped post-harvesting processing. The experiment was carried out following a randomized block design with three replications, in a split-plot scheme, with the plots consisting of five cultivars (Catucaí 2 SL, Catucaí 24/137, Catucaí IAC 44, Arara and Acauã); and the subplots by two processes, pulped and natural cherry. The harvest was carried out manually, harvesting the cherry fruits (ripe) in a sieve. The coffees were processed by the natural and pulped cherry methods. Drying was carried out on a covered suspended terrace, until the grains reached  $11\% \pm 1$  moisture (wet basis, bu). The following evaluations were carried out: sensorial analysis of the beverage according to the SCAA methodology, analysis of grain sieves according to the Official Brazilian Coffee Classification Protocol. The results show that all evaluated cultivars have a great potential for the production of specialty coffees in that studied environment. Differences were observed between cultivars both in the sensory quality of the beverage and in the grain size. Cultivar Arara presents a general average of final beverage grade higher than the other cultivars.

**Key words:** Arabica coffee; coffee sieve size; natural processing; pulped cherries; specialty coffee.

## 1 INTRODUCTION

Brazil stands out as the world's largest producer of coffee, with a total production of 47.72 million processed bags (60kg) in 2021, this commodity has a great importance in international transactions where Brazil is the largest exporter worldwide. The Southeast region has led the Brazilian production with participation in more than half of the national production, with around 40.52 million processed bags. The state of Espírito Santo produced 14.17 million processed bags in 2021 with a production area of 400,442 hectares (Companhia Nacional de Abastecimento - CONAB, 2022).

The production of specialty coffees seeks to provide less vulnerability to the producer in relation to market uncertainties, mainly through the addition of value to this bean, reduction of intermediaries in the commercialization, and increase of exploitation to a growing demand related to the consumption of coffees. So, it is possible to have greater independence within the coffee chain, obtaining greater profits and achieving greater sustainability, being an excellent option for producers who cannot compete on a large scale (Trienekens, 2011; Nicoleli et al., 2015; Clay et al., 2018).

Thus, there has been an increase in the search for cultivars that, in addition to presenting characteristics of resistance to the attack of pests and diseases, have a high potential for beverage quality, in view of the sensory differences that may exist between the genotypes most used today (Fernandes et al., 2020).

The association and interactions of genetic, environmental factors and processing methods directly influence the sensory quality of the beverage, promoting interference in the physical and chemical part of the coffee bean. However, information covering the potential related to the sensorial and physical quality of the Arabica coffee cultivars that has been recommended in Brazil is poor. Adding, these new recommended cultivars need more research aiming to evaluate and characterize these factors, particularly in the state of Espírito Santo where coffee has grown in different soil and climate conditions in to geographic indication regions (Freitas et al., 2020).

Recent studies have demonstrated variations in the potential for obtaining improved sensorial quality and grain size of new Arabica coffee cultivars under highland soil and climatic conditions. The size of the coffee bean has been one

of the parameters evaluated, and this characteristic has been associated with the sensorial quality of the beverage; together these parameters may be important factors in choosing the best cultivar (Krohling et al., 2018).

In addition, choosing the best way to post-harvest processing of Arabica coffee beans provides positive results in several aspects, including physiological and sensory issues. In this way, the evaluation of different processing methods, and their associations with other post-harvest managements, can help to obtain better quality coffee beverages (Ameyu, 2017; Alves et al., 2017).

With the information generated in this work, farmers will be able to better select Arabica coffee cultivars, being able to relate these to the processing methods, grain granulometry and the sensorial quality of the beverage, improving more economic sustainability in the production of specialty coffees. Therefore, this study aimed to evaluate the sensorial quality of the beverage and grain size of five Arabica coffee cultivars, submitted to natural and pulped post-harvest processing.

## 2 MATERIAL AND METHODS

The experiment was carried out in the experimental unit of INCAPER - Instituto Capixaba de Pesquisa Técnica Assistência Técnica e Extensão Rural, located in the municipality of Santa Teresa - ES (19°49'23" S and 40°45'50" W, 780 m altitude).

The region is characterized by a sloping topography with an average annual rainfall of 1,491.6 mm, with seasonality divided into two periods, a rainy one (October to April), which corresponds to 80.4% of the total accumulated annual rainfall for the region, and a drier one (May to September), which represents 19.6% of the accumulated total. The average annual temperature is 19.5 °C, where the highest averages are observed in February (22.1 °C), and the lowest averages in July (16.4 °C).

The coffee crop were planted with spacings of 2.5 meters in rows and 1.0 meters between plants. The implementation and conduct of the experiment were in a randomized block design with three replications, in a split-plot scheme, with the plots

consisting of five cultivars and the sub-plots represented by two post-harvest processing methods, natural and peeled cherry.

The studied cultivars in this experiment are described in Table 1, with their respective characteristics related to fruit color, maturation cycle, rust resistance and genealogy.

Ripe beans were harvested selectively and manually at the cherry stage (20 L/plot) in the useful five central plants of the plots using sieves. After the harvesting process, the coffee beans from each plot were washed separately. The procedure took place in a PVC' buckets with a capacity of 20 L, separating and removing the float-type fruits and other impurities (leaves, sticks, among others). Soon after, the ripe fruits were separated, where 5.0 L of mature coffee from each plot were destined to compose the natural processing, being already sent to the suspended terrace and, thus, starting the drying process. Another 5.0 L of the mature coffee per plot were send to compose the pulped cherry processing. A coffee huller with a capacity of 500 L per hour (PaliniAlves<sup>®</sup>) was used, where, after removing the husk, the samples were taken to PVC buckets for the beginning of spontaneous fermentation, remaining for 24 h with a proportion of water of 30% in relation to the volume of the coffee bean, removing the float-type fruits. Soon after, the samples were washed with water to remove the mucilage, and then sent to the suspended terrace.

The samples were dried on a suspended terrace with a plastic cover, and the control adopted was as follow: i.) pulped processing: on the first day the coffee beans were spread in thin layers of 7 L per m<sup>2</sup>; on the second day this layer was doubled to 14 L per m<sup>2</sup>; on the third day the beans were spread in 3 cm layers; from the fifth day onwards, the beans were dried in 5 cm layers; ii.) natural processing: on the first day the beans were spread in layers of 14 L per m<sup>2</sup>; on the fourth day, the beans were spread in 5 cm rows; on the sixth day the beans were spread in rows of 6 cm, from the sixth day onwards the beans were spread in rows of 8 cm. In both processes the beans was kept on the suspended terrace until reaching 11% ±1 of humidity (wet basis, bu). During the drying period, the beans was turned 10 to 12 times throughout each day, maintaining constant hygiene in the place.

**Table 1:** Description of the main characteristics of the Arabica coffee cultivars.

Coffee cultivar	Fruit color	Maturation Cycle	Rust Resistance	Genealogy
Catucaí 2 SL	Yellow	Medium	MR	Natural cross between Icatu and Catuaí cultivars
Catucaí 24/137	Yellow	Medium	MR	Natural cross between Icatu and Catuaí
Catuaí IAC 44	Red	Medium	S	Artificial cross between the cultivars Caturra Amarelo IAC 476-11 and Mundo Novo IAC 374-19
Arara	Yellow	Late	AR	Natural cross between Icatu and Sarchimor cultivars
Acauã	Red	Medium	AR	Cross between the cultivars Mundo Novo IAC 388-17 and Sarchimor IAC 1668

Source: Adapted from Carvalho (2008), and Matiello (2020). Legend: MR - Moderately Resistant; S - Susceptible; AR – Highly Resistant.

After drying, the samples were stored for a period of 30 days, using packages with a high protection barrier (Grainpro<sup>®</sup>), on wooden pallets in bins. After this time, the samples were processed using equipment with a capacity of 50 kg per hour (PaliniAlves<sup>®</sup>). The processed samples were packed in plastic bags with a capacity of 500 g, and later sent to the Incaper coffee sensory and physical analysis laboratory (CECAFES), located in the municipality of Venda Nova do Imigrante - ES, where the sensorial and physical analysis of the beans were carried out.

In the laboratory, the samples were coded and sent to the sieving processes using sieve 16 and above, seeking standardization, and after that, the selection of the grains was carried out to remove the defects (Ribeiro et al., 2017; Ribeiro et al., 2018). Then, 100 g of each sample were weighed for roasting and subsequent sensorial evaluations.

The TP2-Leogap sample roaster (Probat<sup>®</sup>) was used to roast the bean samples (100 g) which, according to the protocol established by the (Specialty Coffee Association of America - SCAA, 2015), provided a coloration of 58 points on the scale. Agtron for whole grain and 63 points for ground grain, with a tolerance of  $\pm 1$  point. Roasting was performed within a maximum period of 24 h before tasting, where the roasting point was visually determined using a color classification system with standardized discs (SCA/Agtron Roast Color Classification System). The temperature and roasting time were monitored by thermometers and timers, respectively, respecting the time range between eight and 12 min. The beans characterized as “quakers” were removed from the roasted sample, avoiding their derogatory characteristics in the sensorial attributes.

The grinding of the roasted samples was carried out in a mill (Carmomaq<sup>®</sup>) with adjustment so that the size of the ground particles passed 70% of the volume in a sieve with holes of 20 mesh. The equipment was properly cleaned and a small volume of the sample to be milled was crushed to avoid contamination with residues from previously milled samples. The ground samples were distributed into five cups with a concentration of 8.25 g of ground coffee in 150 mL of water, reaching the midpoint of the equilibrium graph, essential for obtaining the Golden Cup (SCAA, 2015).

For sensory evaluations, methodologies established by SCAA were adopted, in which five accredited professionals for the evaluation of special coffees participated in the process, assigning grades to the following characteristics: fragrance and aroma; flavor; finalization; acidity; body; balance; uniformity; clean cup: with the identification of defects in the drink, which lead to a loss of quality, each defective cup was assigned a penalty of two points. Defects were evaluated in two ways, in addition to the clean cup item: mild, when it had an unpleasant taste (two points penalty per cup); severe, when it presents unacceptable sensory characteristics (penalty of four points

per cup). After evaluating the defects, both were added (light and severe) and the value found was subtracted from the total sum obtained after the sensory evaluation; Sweetness and general impression.

The attributes fragrance/aroma, flavor, finish, acidity, body, balance and general, received scores from six to ten with intervals of  $\frac{1}{4}$  point (0.25) between the numerical values, according to the SCAA (2015) methodology (Table 2). To the attributes sweetness, uniformity, and clean cup, the scores were distributed from zero to ten, with numerical intervals of two points.

**Table 2:** Quality scale according to SCAA protocol.

Good	Very Good	Excellent	Exceptional
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

Source: Adapted from SCAA (2015).

Therefore, after evaluating each attribute separately, the sum was performed, where Table 3 shows the possible descriptions of coffee quality.

**Table 3:** Key-result to describe the final quality of the coffee bean samples.

Total Score	Description	Rating
90 – 100	copy	Specialty Rare
85 - 89.99	Great	Specialty Origin
80 - 84.99	Very good	Premium
<80	Below Specialty Quality	Below Premium

Source: Adapted from SCAA (2015).

The following steps were used in the process of granulometric classification of the coffee beans: 100 g of processed coffee beans from each plot were weighed; afterwards, each weighed sample was submitted to a set of sieves, for the evaluation of flat grains in the following order: sieves 19; 18; 17; 16; 15; 14; 13, and bottom. Thus, the grains were grouped in sieve 17 and larger were considered coarse sieve, sieve 15-16 were considered medium sieve and sieve 14 and smaller classified as fine sieve (Brasil, 2003). The grains of each group were weighed on a digital scale with a precision of 0.1 g Scale model, in order to determine the percentage.

For the statistical analyses, were performed analyzes of variance of the final beverage grade and the percentage of coarse sieves, and the means were grouped using the Scott-Knott cluster test at a probability level of 5%. In addition, principal component analyzes were performed to group

the coffees according to their sensory attributes and grain size through visual examinations in graphic dispersions. For statistical analyses, the “easynova” and “Factoshiny” packages of the R program were used (R Core Team, 2022).

### 3 RESULTS

The results show that all evaluated cultivars showed potential for the production of specialty coffees, reaching scores above 85 points, thus falling into the Specialty Origin category (SCAA, 2015). With this, there is great potential of the environment and of the evaluated cultivars, for the production of specialty coffees. Cultivar Arara obtained the highest average of final beverage grade (86.88), while cultivars Catuaí IAC 44 and Acauã presented the lowest averages (85.00 and 85.50, respectively) when compared to the other cultivars. When comparing the different processing methods, although no significant difference was observed, the performance of the cultivars varied according to the processing submitted. The cultivars that presented the highest averages of sensorial note of beverage for the pulped processing method were Arara (86.83), Catucaí 2SL (86.17), and Catucaí 24/137 (85.92). In natural processing, the cultivars Arara (86.95) and Catucaí 24/137 (86.42) had the highest averages (Table 4).

**Table 4:** Averages of final grade of beverage evaluated in five cultivars of *Coffea arabica* L. (Catucaí 2SL, Catucaí 24/137, Catuaí IAC 44, Arara e Acauã) for the pulped and natural cherry processing method.

Coffee cultivar	Post-harvesting processing methods		Average
	Pulped cherry	Natural	
Catucaí 2SL	86.17 aA <sup>(1)</sup>	86.08 aA	86.13 b
Catucaí 24/137	85.92 aA	86.00 aA	85.96 b
Catuaí IAC 44	85.08 bA	84.92 bA	85.00 c
Arara	86.83 aA	86.92 aA	86.88 a
Acauã	85.25 bA	85.75 bA	85.50 c
Average	85.85 A	85.93 A	
General average	85.89		
CV (%)	0.55		

Means grouped by the same lowercase letter in the column and uppercase in the rows do not differ from each other by the Scott-Knott test ( $p < 0.05\%$ ).

The cultivars Catucaí 24/137 (70.67%) and Catuaí IAC 44 (70.33%) showed the highest averages of coarse sieve in the pulped processing. Cultivar Arara (49.67%), on the other hand, presented coarse flat sieve averages lower than the other cultivars in this processing system. The highest averages of coarse sieve in the natural processing were observed in the cultivar Catucaí

24/137 (75.33%), superior to the other cultivars, while the cultivar Catucaí 2SL presented an average of 59.67%, being lower in this processing method. When comparing the post-harvest processing type, we may observe that the cultivar Acauã presented highest average of coarse sieve in the pulped when compared to the natural processing. On the other hand, best performances of the cultivars Catucaí 2 SL e Catucaí 24/137 were noted on the natural then in the pulped process (Table 5). The coarse sieve of the other cultivars Arara and Catuaí IAC 44 were similar when submitted to the two processing methods.

**Table 5:** Averages of the percentages of the sieve characteristic 17 and above, evaluated in five cultivars of *Coffea arabica* L. (Catucaí 2SL, Catucaí 24/137, Catuaí IAC 44, Arara e Acauã) for the pulped and natural cherry processing method.

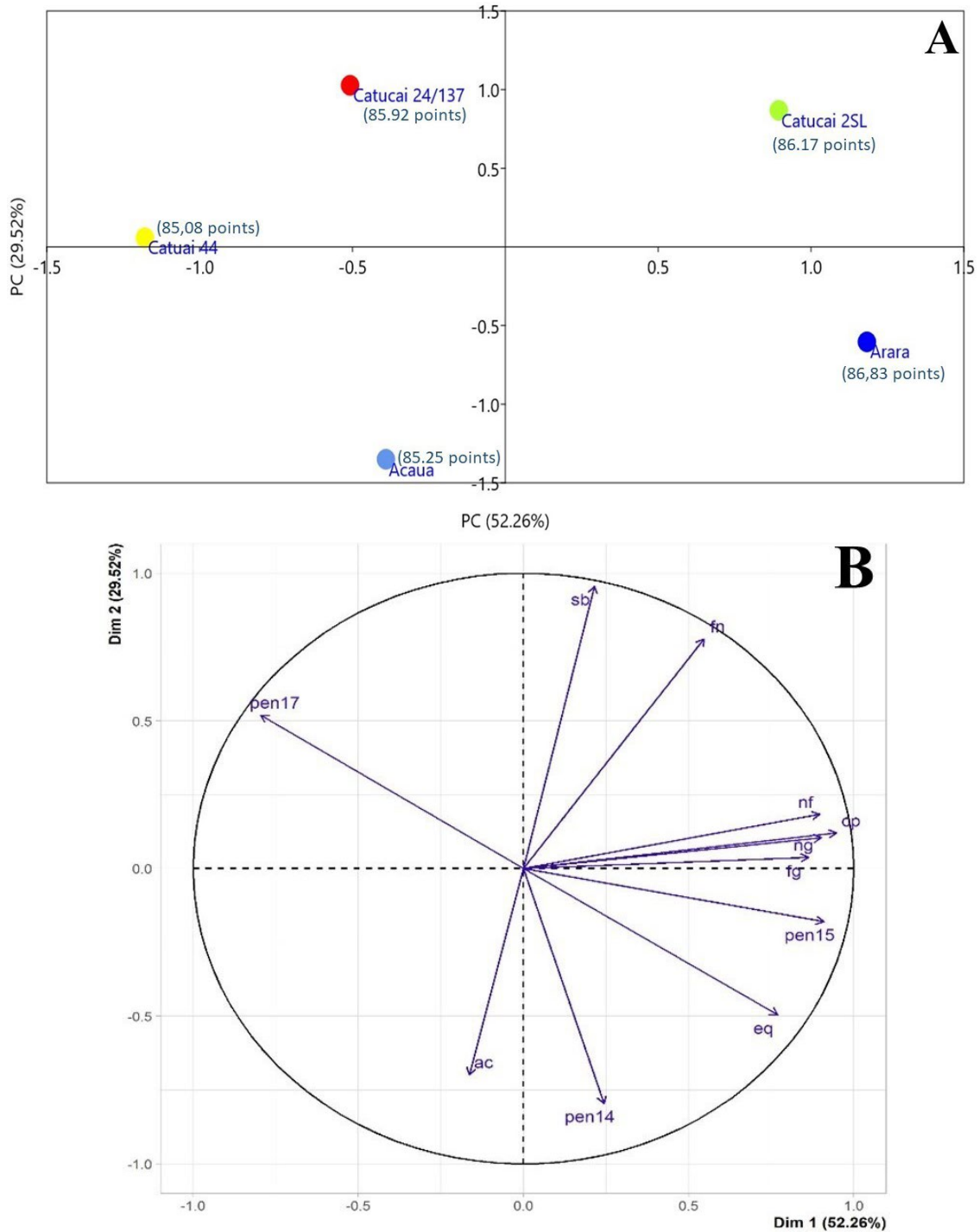
Coffee cultivar	Post-harvesting processing methods			
	Pulped cherry		Natural	
Catucaí 2SL	52.33	bB <sup>(1)</sup>	59.67	cA
Catucaí 24/137	70.67	aB	75.33	aA
Catuaí IAC 44	70.33	aA	69.67	bA
Arara	49.67	cA	50.67	dA
Acauã	52.00	bA	48.00	eB
Average	59.00		60.67	
General average	59.83			
CV (%)	2.06			

<sup>(1)</sup> Means grouped by the same lowercase letter in the column and uppercase in the rows do not differ from each other by the Scott-Knott test ( $p < 0.05$ ).

For the pulped processing, to group the coffees of the five cultivars, regarding the sensorial characteristic of the beverage and size of the grains, the first two principal components (dimensions) were used. Thus, according to Figure 1, it is noted that the principal components, PC1 and PC2 (dimensions), absorbed 81.78% of the existing variations in the original characteristics, with PC1 (dimension 1) with 52.26% and PC2 (dimension 2) with 29.52%.

Thus, it appears that all attributes evaluated in the sensorial analysis of beverage were strongly associated, as shown by the acute angles formed between the vertices. The grain size, on the other hand, showed a low association with the sensorial attributes of quality.

In addition, the sensorial attributes were associated with the cultivars that presented the highest final average of beverage note. The cultivar Arara presented the highest average of final note of beverage and consequently greater association with the sensorial attributes of quality. While the cultivar IAC 44 was the one that presented the lowest final beverage average and the lowest association with the sensorial attributes of the beverage.



**Figure 1:** Scatter diagram in relation to the first two principal components, obtained from sensory characteristics and grain size of five cultivars at 780 m altitude for pulped cherry processing method. Variables: cp = body; fn = finalization; sb = flavor; nf = final grade; fg= fragrance; ng = overall grade; eq = equilibrium; pen17 = sieve 17 and above; pen15 = sieve 15-16; pen14 = sieve 14 and below. Cultivars: C2sl = Catucaí 2SL; C24137 = Catucaí 24/137; CalAC44 = Catuai IAC 44; Arara and Acauã.

The cultivars Catucaí 24/137 and Catuai IAC 44 showed greater association with the percentage of sieve “17 and above”, while the cultivar Catucaí 2SL was associated with the average sieve, 15-16. The cultivar Acauã, on the other hand, was the one that showed

the highest association with fine sieve, 14 and smaller (Table 5).

The disposition of the cultivars in the pulped cherry processing method in the principal components (Figure 1A) can be explained by the fact that the sensory variables (vectors)

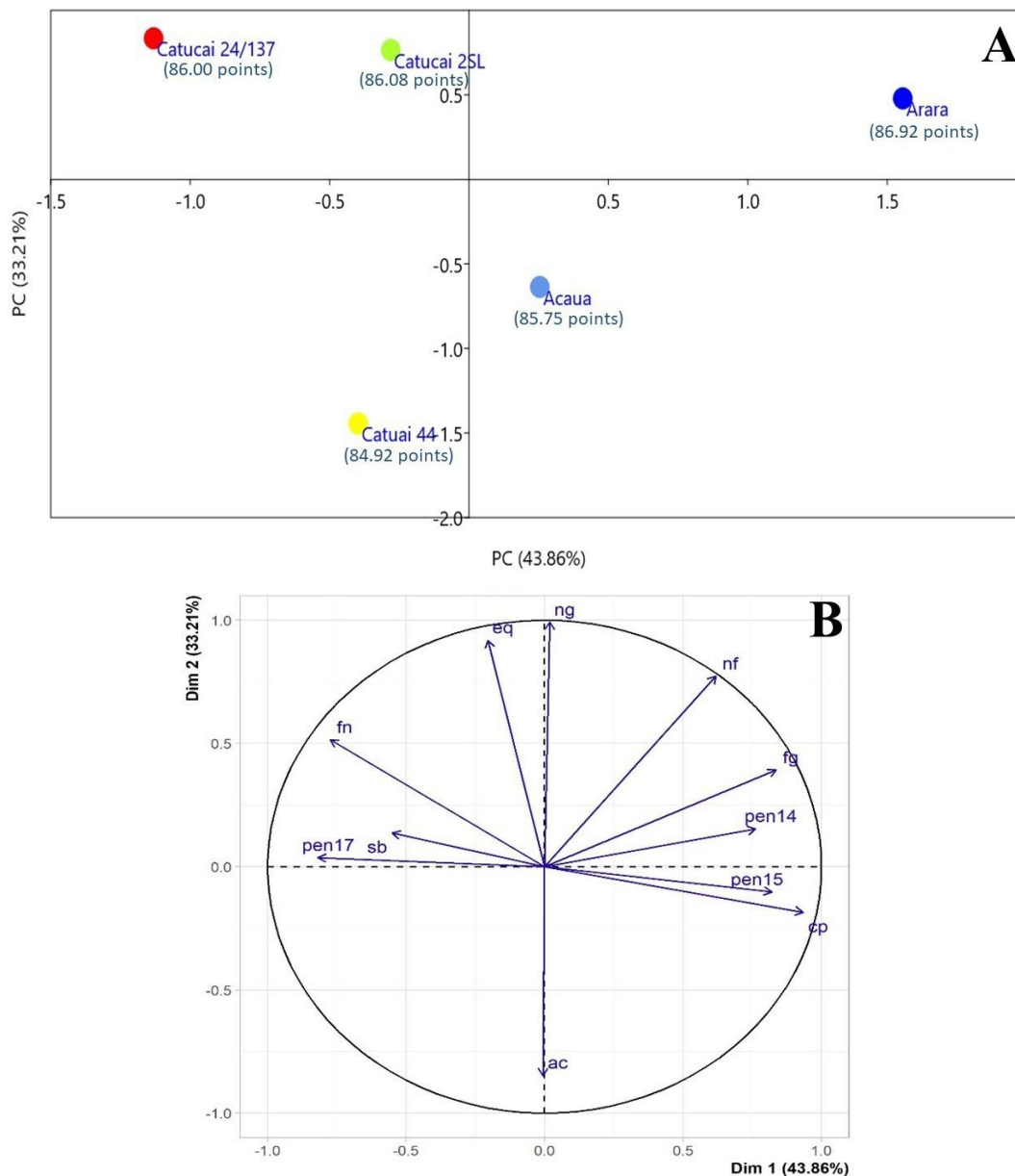


(fg, ng, nf, fn, cp, eq, and sb) (Figure 1B) gave the cultivars Arara, Catucaí 2SL and Catucaí 24/137 the highest final grades (Table 4). It can be observed that these vectors (Figure 1B) were found in the quadrants to the right of component 1, the same quadrants of these cultivars (Figure 1A). Likewise, it was observed that the cultivars with the lowest scores were located in the quadrants to the left of component 1 (Figure 1A, 1B). The cultivar Catucaí 44 with the lowest sensory score (Table 4), being positioned to the left of component 1 (Figure 1A).

To group the coffee cultivars in the natural processing, the first two principal components (dimensions) were also

used, analyzing the sensorial characteristic of the beverage and the size of the grains. The principal components, CP1 and CP2 (dimensions), absorbed 77.07% of the existing variations in the original characteristics, being CP1 (dimension 1) with 43.86% and CP2 (dimension 2) with 33.21% (Figure 2).

As observed in the pulped processing, it appears that all attributes evaluated in the beverage sensory analysis were strongly associated. In addition to the grain size, it also shows a low association with the sensorial attributes of quality.



**Figure 2:** Scatter diagram in relation to the first two principal components, obtained from sensory characteristics and grain size of five cultivars at 780 m altitude for natural processing method. Variables: cp = body; fn = finalization; sb = flavor; nf = final grade; fg = fragrance; ng = overall grade; eq = equilibrium; pen17 = sieve 17 and above; pen15 = sieve 15-16; pen14 = sieve 14 and below. Cultivars: C2sl = Catucaí 2SL; C24137 = Catucaí 24/137; CalAC44 = Catuaí IAC 44; Arara and Acauã.

The sensory attributes were also associated with the cultivars that presented the highest final average of beverage note. The cultivar Arara was also the one that presented the highest average of final beverage grade, and the highest association with the sensorial attributes of quality. In addition, the cultivar IAC 44 had the lowest final beverage average, and the lowest association with the beverage's sensory attributes.

The cultivar Catucaí 24/137 was the most associated with the percentage of sieve "17 and above", while the cultivars Acauã and Catucaí 2SL were associated with medium sieve, 15-16 and fine sieve, 14 and smaller.

The disposition of the cultivars associated with the in the natural cherry processing method in the principal components (Figure 2A) can also be explained by the fact that the sensory variables (vectors) (fg, ng, nf, fn, eq, and sb) (Figure 2B) gave the cultivars Arara, Catucaí 2SL and Catucaí 24/137 the highest final grades (Table 4). It can be observed that these vectors (Figure 2B) were found in the up quadrants of component 2 (33.21% of the absorption of the variability of the original variables), the same quadrants of these cultivars (Figure 2A). Likewise, it was observed that the cultivars with the lowest scores were located in the quadrants to the bottom of component 2 (Figure 2A, 2B). The cultivar Catucaí 44 was also the lowest sensory score (Table 4), being positioned to the bottom of component 2 (Figure 2A).

The analysis of the principal components (Figure 1B and 2B) showed that the Catucaí 44 and Acauã cultivars had more pronounced acidity than the others, with this sensory attribute being more prominent in the natural cherries processing method.

## 4 DISCUSSION

The highest averages associated with the cultivar Arara in terms of the sensory quality of the drink may be related to its genealogical origin, which has as its constituent the cultivar Icatu, which comes from the interspecific hybridization between *C. canephora* and *C. arabica* Bourbon. Since the transfer of *C. canephora* genes with greater adaptation to environmental conditions can contribute to favoring plant physiology and enabling better beverage quality (Sobreira et al., 2016). In addition, the cultivar Bourbon is often associated with the sensory quality of the drink, constituting some lines of research (Ferreira et al., 2013; Ribeiro et al., 2017; Ferreira et al., 2021).

The results of this study confirm those of several other studies that report the genetic potential of the species *Coffea arabica* L. for the production of high sensory quality coffees (Ribeiro et al., 2016; Sobreira et al., 2016; Barbosa et al., 2019; Fernandes et al., 2020). In addition, the differences found between the cultivars show that, even being originated from a narrow genetic base, the species presents variations in the sensory aspect, which may be added to genetic improvement

programs for this trait (Chalfoun et al., 2013; Ribeiro et al., 2016; Fernandes et al., 2020).

Regarding physical quality, the results found in this study corroborate a study carried out by Krohling et al. (2018) who evaluated the adaptation of arabica coffee cultivars in the mountain region of Espírito Santo, Brazil, observing differences as a function of grain size. In this way, it is possible to select genotypes with greater uniformity of grain size, and higher percentages of chato graúdo sieves (Pereira et al., 2013; Rodrigues et al., 2014; Cardoso et al., 2016), having in view of the fact that obtaining coffee beans from high sieves can promote greater aggregation of value to the product, in addition to being related to greater productivity and appreciation of the product in the international market (Pereira et al., 2013; Borém et al., 2020). In a study carried out with progenies from the crossing of Catucaí and Icatu cultivars and descendants of Híbrido de Timor, the progenies that presented the highest averages for the sieve characteristic "17 and above" were those that also presented higher productivity (Pinto et al., 2012).

The differences found in the characteristic of sieve 17 and above between the processing methods can be explained by the variations in the physical-chemical composition of the coffee beans when subjected to different post-harvest processing, which can affect the integrity of the cell membranes of beans and fruits, contributing to the leakage of constituents from the grains (Oliveira et al., 2013). Likewise, the removal of float-type fruits during the wet processing, after the peeling of the fruits and before the beginning of the spontaneous fermentation process, may have interfered in the results.

Therefore, the principal components showed that the cultivar influences the sensory attributes of the coffee beverage and the granulometry. However, by adopting good agricultural practices in the harvest and post-harvest phases, it is possible to produce coffees of sensory and physical quality, ensuring greater sustainability for coffee production (Carvalho et al., 2016; Zaidan et al., 2018; Pimenta, 2020).

## 5 CONCLUSIONS

The five evaluated cultivars have potential for the production of specialty coffees in the region of Santa Teresa – Montanhas do Espírito Santo' geographic indication region, using natural and pulped post-harvest process.

The cultivars Arara, Catucaí 2SL and Catucaí 24/137 presented the final beverage grade superior to the others using the pulped processing. In natural processing, the cultivars Arara and Catucaí 24/137 stand out in terms of this characteristic.

The Arara cultivar stood out in relation to the others in the average of the two types of post-harvest processing. There were variations between cultivars in terms of the potential for the production of coarse sieve coffees 17 and above in the different processing methods evaluated.

The disposition of the cultivars in the principal components can be explained by the fact that the sensory variables gave to the cultivars Arara and Catucaí 2SL, and Catucaí 24/137 the highest final grades. The analysis of the principal components showed that the Catucaí 44 and Acauã cultivars had more pronounced acidity than the others, with this sensory attribute being more prominent in the natural cherries processing method.

## 6 AUTHORS' CONTRIBUTION

RDA wrote the manuscript, performed the experiment, analyzed the data, supervised the research, reviewed and final approval of the work, FTA co-wrote the article, planned the experiment, performed the experiment, performed statistical analyses, supervised the research, reviewed and final approval of the work, PAML co-wrote the article, performed statistical analysis, reviewed and final approval of the work, JFTA supervised the research, review and final approval of the work, CAK supervised the research, review and final approval of the work, MJF supervised the research, review and final approval of the work, RCG supervised the research, review and final approval of the work, RSD performed the experiment, CFV performed the experiment, HAM performed the experiment, CUZ performed the experiment, DBV performed the experiment.

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