

Effects of lactic acid bacteria and yeast inoculation on the sensory quality of *Coffea arabica*

Claudia Milena Amorocho-Cruz^{1*} , Paloma Andrea Tarazona-Sánchez² ,
Wilmer Licerio Ladino-Garzon³ 

¹Universidad Surcolombiana, Programa de Ingeniería Agrícola, Laboratorio Microbiología de Alimentos y Biología Molecular. Neiva, Huila, Colombia

²Federación Nacional de Cafeteros de Colombia, Comité de Cafeteros del Huila. Neiva, Huila, Colombia

³Universidad Surcolombiana, Programa de Ingeniería Agrícola, Centro Surcolombiano de Investigación en Café/CESURCAFÉ, Neiva, Huila, Colombia

*Corresponding author: claudiamilena.amorocho@usco.edu.co

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ABSTRACT

We considering that controlled fermentations and inoculation of starter culture enhance sensory characteristics and ultimately impact cup quality. This study examines the influence of lactic acid bacteria (LAB) and yeast inoculation on the fermentation of coffee from the Colombia and Cenicafé 1 varieties. The pulped coffee was distributed: i) T1 control under open-air, ii) T2 without inoculation in a closed environment, iii) T3 inoculated with *Lactiplantibacillus plantarum* in a closed environment, iv) T4 inoculated with *Candida colliculosa* in a closed environment. A completely randomised experimental design was used to evaluate the effect of fermentation time on microbiological parameters, physico-chemical parameters, and sensory evaluation after roasting. Treatments were assessed at the 0 hours and after 24 hours of fermentation. The lactic acid bacteria treatment significantly enhanced the sensory attributes of the Colombia variety, resulting in higher scores for flavor, acidity, and aroma, with pronounced notes of chocolate and dried fruits. However, in Cenicafé 1, spontaneous fermentation yielded better results, highlighting chocolate and caramel notes, suggesting a lower dependence on starter cultures to optimize quality. Fermentation acidity increased across all treatments, as evinced by pH and titratable acidity results, underscoring the efficiency of the fermentation process. The two varieties exhibited distinct responses: Cenicafé 1 showed lower sensitivity to external treatments, while the Colombia variety demonstrated greater dependence on them to achieve higher-quality sensory profiles. These findings provide valuable insights for tailoring fermentation practices according to coffee variety and desired sensory outcomes.

Key words: Microbial inoculation; yeast; starter cultures; coffee fermentation; *Lactiplantibacillus plantarum*.

1 INTRODUCTION

Colombia is one of the world's leading coffee producers and exporters, ranking third after Brazil and Vietnam (Muzaifa; Abubakar; Nilda, 2024). The department of Huila, Colombia, is the country's top coffee-producing region, making a significant economic contribution as the leading agricultural product in terms of job creation and export value (Cerquera; Pérez Gómez; Sierra, 2020). Coffee from Huila, Colombia, has been recognized for years as a specialty coffee. It has been previously established through empirical evidence that lactic acid bacteria and yeast are present in the coffee fruit and during the fermentation process. The activity exerted by these organisms is yet to be fully elucidated. To this end, isolates have been obtained, identified through biochemical tests, and used as starter cultures to relate this information to the attributes found in the cup of coffee.

The final quality of coffee is determined by its processing method, which can be wet, dry, or semi-dry, each designed to enhance its market value (Muzaifa; Abubakar; Nilda, 2024). Traditionally, Colombia has relied on wet processing, producing the globally renowned smooth, washed coffees. However, semi-dry processing has gained traction, imparting distinctive sensory characteristics that influence the end cup profile. This differentiation serves as a key advantage

in accessing specialized markets (De Melo Pereira et al., 2020).

In the coffee fruit, the mucilage have polysaccharides, glucose, fructose, citric acid, malic acid, free amino acids and phenolic compounds, which can be used as nutrients or co-substrates by bacteria, yeast (Vasileios et al., 2020) and other microorganisms of environment.

Saccharomyces cerevisiae is a widely used yeast in the food industry, and *Candida* species have also proven valuable in cheese production and as starter cultures in the fermentation of coffee, cocoa, vegetables, meat, beer, and wine, as they contribute specific characteristics as they produce key metabolites and enzymes (De Melo Pereira et al., 2022).

Lactic acid bacteria and yeasts are used to enhance beneficial endogenous microorganisms in coffee, improving its overall sensory profile through symbiotic interactions (Cassimiro et al., 2023). This process also plays a crucial role in controlling ochratoxin A (OTA)-producing molds (Meftah et al., 2018). Notably, *Lactiplantibacillus plantarum* has been studied in robusta coffee for its antagonistic effect against *Aspergillus carbonarius*, further highlighting its potential in coffee fermentation (Diossou et al., 2011). In Colombia, De Oliveria Junqueira et al. (2019) found that in coffee from Buesaco, Nariño, Colombia to 1959 m altitude, LAB was represented by *Leuconostoc* and *Lactobacillus*, yeast as *Pichia nakasei* throughout the fermentation process. The microorganisms

present yielding metabolites as lactic acid, acetaldehyde, alcohols, other organic acid, aldehydes, terpenes, esters, phenols, ketones and hydrocarbons (Feng et al., 2016). These microbial groups may imparts flavours that produced clues to the terroir Colombian coffees. The microbial ecology of a coffee fermentation can be highly variable; for example samples of coffee from Tanzania in Africa found *Pichia kluyveri*, *Pichia anomala*, *Hanseniaspora uvarum*, *Kluyveromyces marxianus*, *Candida pseudointermedia*, *Issatchenkia orientalis*, *Pichia ohmeri* and *Torulaspora delbrueckii* (Masoud et al., 2004). According to Vasileios et al. (2020) coffee postharvest depending on the technological parameters or geographical conditions.

Therefore fermentation is a key stage in coffee post-harvest processing, where microorganisms play a crucial role in transforming the sugars and other compounds in the fruit's mucilage. This process not only aids in mucilage removal from the beans but also had a significant impact on the sensory profile, helping develop unique flavor characteristics (Pereira, B. A. P. et al, 2020). In addition, a complex succession of transformations during the processing of seeds to roasted coffee will inevitably influence the in cup attributes of coffee (Waters; Arendt; Moroni, 2017).

There are several researches with coffee arabica and robusta using starter cultures using single culture, two mixed and evaluating their effect in a cup quality (Velmourougane et al., 2008; Silva et al., 2013; De Melo Pereira et al., 2014; De Melo Pereira et al., 2015) for standardizing the sensorial profiles (Vasileios et al., 2020). Research has shown that microbial inoculation and the spontaneous anaerobic conditions created by microbial metabolism, enhance coffee's sensory quality by positively influencing the fermentative activity of lactic acid bacteria and yeasts (Martinez Jimenez et al., 2025). Mota et al. (2020) demonstrated that flavor and aromatic attributes can be modulated using yeasts as starter cultures in combination with lactic acid bacteria (Meftah et al., 2018) while Bravim et al. (2023) found that coffee inoculated with lactic acid bacteria achieved higher sensory evaluation scores compared to treatments relying solely on native microbiota.

Building on existing research that demonstrates how fermentation enhances coffee's sensory quality during processing, this study evaluates the cup quality of *Coffea arabica* L. varieties Cenicafé 1 and Colombia under different fermentation conditions. Specifically, we assessed the effect of inoculation with *Lactiplantibacillus plantarum* and *Candida colliculosa* on the sensory profile of these *Coffea arabica* varieties.

2 MATERIAL AND METHODS

2.1 Materials

Yeast strain *Candida colliculosa* and lactic acid bacteria strain *Lactiplantibacillus plantarum*, 600 kg of ripe fruits

(*Coffea arabica* L.), buffered peptone water (Merck KGaA, Germany), yeast extract glucose chloramphenicol agar (YGC, Merck KGaA, Germany), Man, Rogosa and Sharpe broth (MRS Broth, Condalab, Spain), Man, Rogosa and Sharpe agar (MRS Agar, Condalab, Spain), shaking incubator (KS 4000i control IKA, Colombia), orbital shaking incubator (TE-4200 Tecnal, Brazil), centrifuge (Ortoalresa Bioproceen 22 R, Spain), refractometer (Milwaukee MA871, USA), portable pH meter (PH 850 Apera Instruments, USA), moisture analyzer (KETT PM 650, Japan), incubator (Thermo Scientific, USA), incubator (Mettler, Germany), anaerobic jar (Oxoid, Spain), laboratory roaster (Prisma Coffee Tech, Prisma DR-1, Envigado, Colombia), electric grinder (Mahlkönig EK43 S, Zurich, Switzerland), statistical software Statgraphics Centurion XVI.I.

2.2 Experimental design

A completely randomized experimental design was carried out for each coffee variety evaluated independently. The objective was to evaluate the effect of fermentation time on microbiological and physicochemical parameters. Two levels of fermentation time were evaluated (0 hours and 24 hours) and the dependent variables analyzed were lactic acid bacteria count, yeast count, total dissolved solids, pH and titratable acidity. All assays were performed in duplicate.

2.3 Selection of Starter Cultures

Coffee samples were inoculated with *Candida colliculosa* (P-CBS-30-2) yeast and the *Lactiplantibacillus plantarum* (F-CBS-18-2) lactic acid bacteria strain, isolated from coffee in previous research both sourced from the Food Microbiology Laboratory's culture collection at the School of Engineering, Universidad Surcolombiana, Neiva, Huila, Colombia. The strains were cultivated separately under controlled conditions: the yeast in peptone water at 30 °C with agitation at 70 rpm (KS 4000i control IKA, Colombia), and the lactic acid bacteria strains in MRS broth at 37 °C with orbital shaking (TE-4200 Tecnal, Brazil) until reaching an optimal optical density of 0.209 (600 nm) and 2.322 (550 nm) absorbance units, respectively (Genesys 180 Thermo Fisher Scientific, USA). The cells were then centrifuged at 6000 rpm for 8 minutes (Ortoalresa Bioproceen 22 R, Spain) and resuspended in sterile water before inoculation.

2.4 Coffee Harvesting and Processing

A total of 600 kg of ripe cherries (*Coffea arabica* L.) were harvested. The fruits were harvested by hand, and subsequently sorted according to their density when immersed in water. The distribution is uniform across both varieties of coffee: 300 kg of the Colombia variety from El Progreso farm (02°38.941' - 075°40.334') at 1,431 m.a.s.l. (Agroecological Zone 2) in the municipality of Íquira, Huila, Colombia, and 300

kg of the Cenicafé 1 variety from Lusitania farm (02°15.457' - 075°30.802') at 1,350 m.a.s.l. (Agroecological Zone 5) in the municipality of Gigante, Huila, Colombia.

The pulped coffee was distributed into three treatments and a control, with duplicates of 20 kg each. The treatments were as follows: a control treatment under open-air conditions typical of coffee farms (T1), a treatment without inoculation in a closed environment (T2), a treatment inoculated with lactic acid bacteria in a closed environment (T3), and a treatment inoculated with yeasts in a closed environment (T4).

2.5 Analytical Determinations

2.5.1 Physicochemical determinations

Temperature, soluble solids (Milwaukee MA871, USA), and pH (Apera Instruments portable, USA) were recorded at the beginning (0 hours) and end of the fermentation stage (24 hours). Following fermentation, the coffee was dried on raised beds until reaching a moisture content of 10% (KETT PM-650, Japan). The samples were then collected in airtight bags to protect them from external moisture and subsequently subjected to physical and sensory analysis.

2.5.2 Microbiological Analysis

Ten grams of mucilage samples were collected during fermentation at 0 and 24 hours and homogenized in 90 mL of buffered peptone water (Merck KGaA, Germany) and Man, Rogosa, and Sharpe (MRS) broth (Condalab, Spain). Serial dilutions were prepared and plated on yeast extract-glucose-chloramphenicol (YGC) agar (Merck KGaA, Germany) and MRS agar (Condalab, Spain). The plates were incubated at 30 °C under aerobic conditions (Thermo Scientific incubator, USA) and at 37 °C under anaerobic conditions (Mettler incubator, Germany) using anaerobic jar for 48 hours (Oxoid, UK). Following incubation, the Colony-Forming Units per milliliter (CFU/mL) were quantified.

2.5.3 Sensory Analysis

Twenty-four hours before sensory evaluation, 500 g of coffee from each treatment were roasted using a laboratory roaster (Prisma Coffee Tech, Prisma DR-1, Envigado, Colombia). The roast level was measured using coffee ground to a standard cupping size, following the 63 Agron Gourmet scale (Lighttells Corp. Ltd., CM100+, Taiwan, China).

The samples were weighed to maintain a predetermined ratio of 8.25 ± 0.25 g per 150 mL of water and ground with an electric grinder (Mahlkönig, EK43 S, Zürich, Switzerland). Sensory evaluation was conducted by a panel of five Q-grade coffee tasters, adhering to the cupping protocol established by the Specialty Coffee Association (SCA).

Ten quality attributes were assessed: fragrance/aroma, flavor, aftertaste, acidity, body, balance, uniformity,

cleanliness, sweetness, and overall score. Evaluations were performed on a scale of 6.00 to 9.75. Each taster assessed five cups per treatment, and the final score was determined by summing the scores of the evaluated attributes (Ladino; Barrios-Rodríguez; Amorocho-Cruz, 2024).

2.5.4 Statistical Analysis

Microbiological counts, physicochemical parameters, and sensory analysis results were evaluated using a one-way ANOVA in the Statgraphics Centurion XVI.I software. Fisher's LSD test was performed with a significance level of 95%.

3 RESULTS

3.1 Microbiological and Physicochemical Characterization

Table 1 details the microbiological and physicochemical parameters measured in the field for each treatment during a 24-hour fermentation process. Data were recorded at the start and end of fermentation for both Colombia and Cenicafé 1 varieties.

At the end of the fermentation period, microbiological counts were conducted for the different treatments (Table 1). The results indicated that lactic acid bacteria populations were consistently higher than yeast populations, both at the start and end of fermentation.

The microbiota in the Colombia variety samples presented higher counts of both lactic acid bacteria and yeasts at the end of fermentation, except in treatment T2, where yeast counts were higher at the beginning of fermentation. Lactic acid bacteria counts ranged from 7.37 to 7.96 Log CFU/mL at the start and from 8.55 to 9.18 Log CFU/mL at the end of fermentation. Yeast counts ranged from 7.19 to 7.35 Log CFU/mL at the start and from 7.35 to 7.54 Log CFU/mL at the end.

At the start of fermentation, treatments T2, T3, and T4 showed no statistically significant differences in lactic acid bacteria and yeast counts, suggesting that induced anaerobic conditions promoted microbial growth. After 24 hours, the lactic acid bacteria-inoculated (T3) and yeast-inoculated (T4) treatments displayed significantly higher microbial counts compared to the other treatments, indicating that the inoculated microbiota successfully adapted to the fermentation mass, enhancing its growth.

The positive influence of closed systems on microbial development was evident, as these enabled faster adaptation and microbial proliferation. This suggests that fermentation attributes could be modulated in a shorter time due to metabolite production. These conditions correlate with the higher sensory scores obtained for the Colombia variety in closed treatments (T2, T3, and T4), as shown in Table 2, where they are classified as 'very good' specialty coffees.

Table 1: Physicochemical variables and viable cell count (Mean $\pm$ SD).

Variety	Parameter	Treatment	0 h	24 h	P value
Colombia	LAB (Log UFC/mL)	T1	7.37±0.11a	8.55±0.30c	0.0000
Cenicafé 1			8.11±0.48abc	8.09±0.19abc	0.0000
Colombia	Yeasts (Log UFC/mL)		7.19±0.09a	7.40±0.12c	0.0000
Cenicafé 1			6.80±0.14b	6.29±0.41a	0.0000
Colombia	Soluble solids (°Brix)		14.40±0.52d	13.76±0.15d	0.0000
Cenicafé 1			13.70±0.10b	13.80±0.10b	0.0070
Colombia	pH		4.61±0.01e	3.93±0.01c	0.0000
Cenicafé 1			5.91±0.01e	4.26±0.03c	0.0000
Colombia	Acidity		0.81±0.03a	1.54±0.00b	0.0000
Cenicafé 1			0.56±0.02a	1.53±0.12b	0.0000
Colombia	LAB (Log UFC/mL)	T2	7.75±0.36b	8.76±0.20c	0.0000
Cenicafé 1			8.28±0.16bcd	7.81±0.19a	0.0000
Colombia	Yeasts (Log UFC/mL)		7.35±0.22b	7.35±0.19c	0.0000
Cenicafé 1			6.70±0.16b	6.82±0.25b	0.0000
Colombia	Soluble solids (°Brix)		12.40±1.18c	10.88±1.04b	0.0000
Cenicafé 1			13.73±0.16b	13.38±0.56b	0.0070
Colombia	pH		4.56±0.04de	3.65±0.02a	0.0000
Cenicafé 1			5.77±0.09d	3.82±0.09a	0.0000
Colombia	Acidity		0.85±0.13a	2.31±0.10d	0.0000
Cenicafé 1			0.49±0.02a	1.98±0.14c	0.0000
Colombia	LAB (Log UFC/mL)	T3	7.96±0.35b	9.18±0.33d	0.0000
Cenicafé 1			7.98±0.30ab	8.39±0.33cd	0.0000
Colombia	Yeasts (Log UFC/mL)		7.21±0.18b	7.42±0.19d	0.0000
Cenicafé 1			6.77±0.17b	7.34±0.20c	0.0000
Colombia	Soluble solids (°Brix)		14.00±0.42d	12.01±0.57c	0.0000
Cenicafé 1			13.63±0.08b	13.63±0.59b	0.0070
Colombia	pH		4.53±0.04d	3.84±0.02b	0.0000
Cenicafé 1			5.89±0.03e	4.22±0.12c	0.0000
Colombia	Acidity		0.84±0.03a	1.85±0.04c	0.0000
Cenicafé 1			0.59±0.12a	1.41±0.10b	0.0000
Colombia	LAB (Log UFC/mL)	T4	7.89±0.24b	8.79±0.35c	0.0000
Cenicafé 1			7.87±0.20a	8.47±0.35d	0.0000
Colombia	Yeasts (Log UFC/mL)		7.30±0.28b	7.54±0.22c	0.0000
Cenicafé 1			6.61±0.21ab	6.69±0.15b	0.0000
Colombia	Soluble solids (°Brix)		11.83±1.25bc	8.83±0.73a	0.0000
Cenicafé 1			13.73±0.05b	12.81±0.67a	0.0070
Colombia	pH		4.57±0.04e	3.82±0.01b	0.0000
Cenicafé 1			5.91±0.08e	4.11±0.06b	0.0000
Colombia	Acidity		0.80±0.02a	2.21±0.11d	0.0000
Cenicafé 1			0.57±0.02a	1.52±0.11b	0.0000

SD - Standard deviation; T1 – Control treatment under open-air conditions typical of coffee farms; T2 – Treatment without inoculation in a closed environment; T3 – Treatment inoculated with lactic acid bacteria in a closed environment; T4 – Treatment inoculated with yeasts in a closed environment. Lowercase letters in the same column (parameters) and row (fermentation times) indicate significant differences between treatments at a 5% significance level ($p < 0.05$) using Fisher's LSD test. The assumptions of the analysis of variance, such as the normality of the residuals and the homogeneity of variance, were verified. For the purpose of evaluating microbiological parameters, the Shapiro-Wilk and Levene's statistical tests were employed, while for physicochemical parameters, the Kolmogorov-Smirnov and Bartlett statistical tests were utilised.

The microbial counts for the Cenicafé 1 variety showed a similar trend to those of the Colombia variety, with lactic acid bacteria populations consistently higher than yeast populations at both the beginning and end of fermentation. Lactic acid bacteria counts increased from the start to the end of fermentation in the inoculated treatments, with values ranging from 7.78 to 8.39 Log CFU/mL in T4 and from 7.87 to 8.47 Log CFU/mL in T3.

Conversely, in treatments T1 and T2, microbial populations declined by the end of fermentation. This trend is likely associated with inoculation, which may enhance microbial adaptability, growth, and competition between microbial genera. In T1, the open system conditions may have kept native microorganisms in an adaptation phase, as no statistically significant differences were observed between the initial and final sampling points, suggesting the activation of aerobic microorganisms.

A similar pattern was observed in T2, where conditions differed only in being a closed system. At the start of fermentation, microbial counts in T2 did not statistically differ from those in T1. However, anaerobic conditions may have aided the activation of native microorganisms requiring anaerobic environments for their metabolic pathways. These microbes may have undergone an adaptation phase or required more time to proliferate, which could explain the decline in microbial load at the end of fermentation under the proposed timeframe.

Yeast populations in the fermentation mass of the Cenicafé 1 variety increased from the beginning to the end of fermentation in treatments T2, T3, and T4, whereas in T1, they declined. This suggests that the closed-system conditions positively influenced microbial proliferation. However, when comparing these results with sensory evaluation, traditional

processing methods appear to be more suitable for this variety in attaining higher cup quality scores (Table 3).

Notably, under induced anaerobic fermentation conditions, both coffee varieties demonstrated the potential to achieve specialty coffee status, as evinced in Tables 2 and 3. However, microbiota composition and final cup quality appear to be influenced by the agroecological characteristics of the regions where the trials were conducted, as well as by the specific coffee variety. This indicates that closed fermentation processes are not always necessary to enhance cup quality scores, as seen for the Cenicafé 1 variety. Furthermore, microbial growth exhibited significant diversity, with microorganisms producing metabolites that could be transferred to the coffee bean, ultimately shaping the sensory attributes of flavor and aroma in the final beverage.

In both the Colombia and Cenicafé 1 varieties, soluble solids progressively decreased throughout the fermentation process (Table 1). This decline is attributed to the consumption of sugars as the primary energy source, primarily by yeasts and lactic acid bacteria, which play a crucial role in the biochemical transformations that shape the development of sensory attributes.

Table 1 also shows a significant decrease in pH across all treatments, with statistically significant differences between the start and end of fermentation. Throughout the process, pH levels remained within an acidic range, indicating optimal conditions for microbial metabolic activity.

In contrast, titratable acidity followed an inverse trend, increasing steadily throughout fermentation. This increase, with statistically significant differences between the initial and final stages, was quantified in terms of chlorogenic acid, a key compound contributing to the sensory attributes of coffee.

Table 2: Attributes, final score, and notes of the Colombia coffee variety according to the specialty coffee association (Mean $\pm$ SD).

Treatment	Flavor	Aftertaste	Acidity	Overall score	Total score	Descriptor	
						Positive	Negative
T1	7.53 $\pm$ 0.29 ^a	7.18 $\pm$ 0.22 ^a	7.40 $\pm$ 0.23 ^a	7.38 $\pm$ 0.38 ^a	82.10 $\pm$ 1.35 ^a	Citrus, panela, brown sugar, plum	Wood, iodine, chemical, straw, nicotine
T2	7.66 $\pm$ 0.35 ^{ab}	7.40 $\pm$ 0.38 ^{ab}	7.56 $\pm$ 0.25 ^{ab}	7.51 $\pm$ 0.20 ^{ab}	82.78 $\pm$ 2.29 ^{ab}	Vanilla, malt, spices, herbal	Ash, bruised fruit, tobacco
T3	7.89 $\pm$ 0.14 ^c	7.61 $\pm$ 0.30 ^b	7.62 $\pm$ 0.23 ^b	7.68 $\pm$ 0.42 ^b	83.91 $\pm$ 1.14 ^{ab}	Chocolate, nuts, caramel, butter	Wood, astringent, vegetal, dry
T4	7.78 $\pm$ 0.23 ^{bc}	7.48 $\pm$ 0.28 ^b	7.56 $\pm$ 0.21 ^{ab}	7.64 $\pm$ 0.27 ^{ab}	83.11 $\pm$ 1.66 ^b	Anise, soy, cinnamos, cloves	Dry, mettalic, bitter
P Value	0.0003	0.3500	0.0006	0.0123	0.0070		

SD - Standard deviation; T1 – Control treatment under open-air conditions typical of coffee farms; T2 – Treatment without inoculation in a closed environment; T3 – Treatment inoculated with lactic acid bacteria in a closed environment; T4 – Treatment inoculated with yeasts in a closed environment. Lowercase letters in the same column indicate significant differences between treatments at a 5% significance level ($p < 0.05$) by Fisher's LSD test. The assumptions of analysis of variance, such as the normality of residuals and the homogeneity of variance, were examined. The Kolmogorov-Smirnov and Levene statistical tests were employed to analyse the attributes and total score, respectively.

Table 3. Attributes, final score, and notes of the Cenicafé 1 coffee variety according to the specialty coffee association (Mean $\pm$ SD).

Treatment	Flavor	Aftertaste	Acidity	Overall score	Total score	Descriptor	
						Positive	Negative
T1	8.17 $\pm$ 0.23b	7.73 $\pm$ 0.21a	7.87 $\pm$ 0.10b	8.03 $\pm$ 0.28b	85.32 $\pm$ 1.00b	Cocoa nibs, caramel, chocolate, berries, plum, winey (ripe fruit)	Vinager, straw, vegetal, dry
T2	7.75 $\pm$ 0.26a	7.60 $\pm$ 0.27a	7.58 $\pm$ 0.18a	7.71 $\pm$ 0.25a	83.46 $\pm$ 1.23a	Panela, roasted corn, brown sugar, molasses, caramel. Sweet overall	Dry, bruised fruit, nicotine, wood
T3	7.78 $\pm$ 0.19a	7.51 $\pm$ 0.30a	7.53 $\pm$ 0.22a	7.69 $\pm$ 0.22a	83.20 $\pm$ 1.06a	Panela, nuts, hazelnut, chocolate, caramel.	Nicotine, tobacco, straw
T4	7.87 $\pm$ 0.17a	7.60 $\pm$ 0.30a	7.58 $\pm$ 0.16a	7.70 $\pm$ 0.25a	83.72 $\pm$ 1.17a	Panela, herbal, guava, apple, raisin, plum, caramel	Dry, straw, wood
P Value	0.0137	0.0249	0.1544	0.0958	0.0880		

SD - Standard deviation; T1 – Control treatment under open-air conditions typical of coffee farms; T2 – Treatment without inoculation in a closed environment; T3 – Treatment inoculated with lactic acid bacteria in a closed environment; T4 – Treatment inoculated with yeasts in a closed environment. Lowercase letters in the same column indicate significant differences between treatments at a 5% significance level ($p < 0.05$) by Fisher's LSD test. The assumptions of analysis of variance, such as the normality of residuals and the homogeneity of variance, were examined. The Kolmogorov-Smirnov and Levene statistical tests were employed to analyse the attributes and total score, respectively.

3.3 Sensory Analysis

Tables 2 and 3 present the ratings of sensory attributes, total scores, and descriptors evaluated by a panel of experts for the Colombia and Cenicafé 1 varieties. Only attributes that showed statistically significant differences (flavor, aftertaste, acidity, and overall score) were included.

The sensory profile of the Colombia variety (Table 2) reveals a consistent trend in treatments T1 and T3. Treatment T3 recorded the highest values across all evaluated attributes with statistically significant differences, suggesting a stronger preference among the sensory panel and greater acceptance of its attributes. Treatment T1, in contrast, exhibited lower values, reflecting reduced sensory acceptance by the evaluators. These two treatments present statistically significant differences from each other, a pattern that holds across all assessed attributes.

This variety's sensory profile varied depending on the treatment, and it is characterized by a diverse range of flavors, including sweetness, chocolatey, fruity, and spicy notes. However, each treatment introduced specific attributes that negatively impacted the coffee's overall quality.

The Cenicafé 1 coffee variety exhibits a contrasting trend to that of the Colombia variety. The highest scores were recorded in treatment T1, associated with spontaneous fermentation (Table 3), showing statistically significant differences compared to the other treatments. In contrast, treatment T3 consistently received the lowest scores across its attributes.

The results obtained for the Cenicafé 1 variety suggest that it has intrinsic qualities that naturally enhance its ability to develop an outstanding sensory profile. This variety

consistently delivers high-quality performance, regardless of the processing method applied, demonstrating remarkable autonomy in expressing its full sensory potential.

4 DISCUSSION

4.1 Microbiological Analysis

The behavior of the analyzed microbiota aligns with the trend described by Evangelista et al. (2014), who reported that bacteria dominate the initial stages of fermentation, followed by yeasts, regardless of the processing method used. This same pattern was reported by Sanchez-Riaño et al. (2024), who found that yeast counts were lower than those of lactic acid bacteria. Importantly, given the induced anaerobic conditions during fermentation for both coffee varieties in this study, the findings are consistent with those of Sanchez-Riaño et al. (2024), who classified coffee under these conditions as 'very good' according to the SCA.

Moreover, significant variability in microorganism growth is evident from the microbial counts obtained. (Table 2). These microorganisms can influence the sensory characteristics of the final beverage by producing metabolites that are transferred to the beans (Elhalis, 2020a; Elhalis, 2020b). This explains why all treatments were classified as specialty coffees, highlighting the potential for scaling up selection trials to enhance the production of high-quality coffees.

4.2 Soluble Solids (°Brix)

Puerta (2012) observed that in waterless fermentation systems, °Brix values decrease exponentially, though at

a decelerating rate. During anaerobic fermentation, the soluble solids content progressively declines over time, suggesting the degradation of sugars and other soluble compounds, likely through specific enzymatic and metabolic activities (Hakim et al., 2024). However, in treatment T1 of the Cenicafé 1 variety, there was a minimal—though not statistically significant—increase. According to Olivares et al. (2019), this phenomenon occurs because, during mucilage degradation, bacteria convert pectic compounds and sugars into organic acids, while yeasts produce alcohols as part of their metabolic activity. This process increases the concentration of dissolved substances in the exudate formed during fermentation.

4.3 Hydrogen Potential and Acidity (pH)

Studies conducted by Puerta (2012) suggest that a final pH between 3.7 and 4.1 is optimal for halting coffee fermentation, as this process involves complex biochemical pathways and the formation of volatile compounds that influence the bean's sensory attributes. These findings align with those of Peñuela-Martínez et al. (2023), who documented a final pH close to 4.0. In the present study, the final pH fell within this range, confirming consistency with the literature. Similarly, Vaz et al. (2023) state that pH progressively decreases as fermentation time advances due to the production of organic acids by lactic acid bacteria.

The rise in acidity during fermentation is primarily attributed to the activity of fermentative bacteria, particularly lactic acid bacteria, which produce lactic acid, as well as acetic acid formed through bacterial activity and alcohol acetification, as noted by Puerta (2015). Moreover, Galarza and Figueroa (2023) demonstrate that acidity increases over fermentation time due to acids released from the degradation of pectin and coffee sugars. This increase in acidity plays a key role in enhancing the sensory characteristics of Colombian coffee, contributing to greater aromatic and flavor complexity.

4.4 Sensory Analysis

The use of mixed cultures, such as the co-inoculation of lactic acid bacteria and yeasts, has been identified as a key factor in increasing the concentrations of desirable volatile compounds, including esters, alcohols, and aldehydes. These compounds play a crucial role in enhancing the sensory attributes of coffee, contributing to fruity and fermented notes that may explain the higher sensory acceptance observed in treatment T3 (Zhao et al., 2024; Rabelo et al., 2024). Furthermore, incorporating lactic acid bacteria during fermentation results in a more complex flavor profile, compared to spontaneous fermentation, leading to improved acidity and overall sensory quality (Rabelo et al., 2024).

The incorporation of lactic acid bacteria not only accelerates fermentation, but also enhances flavor and aroma profiles, resulting in a higher-quality beverage with distinctive sensory attributes. During fermentation, lactic acid bacteria metabolize sugars and other compounds in the coffee pulp (Wang et al., 2019). This underscores the significance of lactic acid bacteria in the fermentation process (Pereira et al., 2020). These findings are reflected in Table 2, where tasters consistently identified distinctive chocolate and nut notes in the analyzed samples.

Peñuela et al. (2023) evaluated fermentation in three coffee varieties and found that, although fermentation patterns were similar across varieties, the sensory quality of Cenicafé 1 showed no significant differences between controlled and spontaneous fermentation. The authors suggest that this finding indicates Cenicafé 1 maintains its high sensory quality regardless of the fermentation method used.

Similarly, in this study (Table 3), treatment T1 achieved the highest scores, surpassing treatments where yeasts and lactic acid bacteria were inoculated and fermented anaerobically. This result suggests that, unlike other varieties, Cenicafé 1, produced at the Lusitania farm, does not depend on external treatments to optimize its quality.

From a sensory perspective, Cenicafé 1 stands out for its well-balanced flavor profile, often characterized by chocolate and caramel notes - attributes that are further enhanced through optimal processing methods (Kitzberger, 2010; Teran, 2024). Moreover, this variety exhibits medium acidity and a full-bodied texture, elements that contribute to a rich and enjoyable sensory experience, highly valued by both consumers and experts (Teran, 2024; Condelli et al., 2022). This trend was confirmed in this study, as tasters consistently identified these distinctive notes across the evaluated treatments (Table 3).

The present study demonstrates that the unique characteristics of coffee vary according to factors such as farm location, coffee variety, agroecological conditions, fermentation method, and drying process. These factors contribute to the production of coffees with diverse characteristics, thereby reaching a diverse audience with varied tastes.

5 CONCLUSIONS

The anaerobic conditions created by closed systems facilitate greater adaptability of both endogenous and inoculated microbiota within the fermentation mass, enhancing cup quality, as observed in the Colombia variety at La Mariana farm. However, for the Cenicafé 1 variety, this process does not improve its sensory characteristics. Instead, it is suggested that the intrinsic properties of Cenicafé 1 allow for the production of high-quality coffee through spontaneous fermentation at Lusitania farm.

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7 AUTHOR CONTRIBUTIONS

Conceptual idea: Amoroch-Cruz C.M.; Methodology design: Amoroch-Cruz C.M.; Ladino-Garzon W.L.; Data collection: Tarazona-Sanchez P.A.; Ladino-Garzon W.L.; Data analysis and interpretation: Tarazona-Sanchez P.A.; Ladino-Garzon W.L., Amoroch-Cruz C.M.; Writing and editing: Tarazona-Sanchez P.A.; Ladino-Garzon W.L.; Amoroch-Cruz C.M.].

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