Exploratory evaluation of dry fermentation of specialty coffee from Nariño-Colombia, -using wet and honey-like methods

Cindy Estefanía Córdoba Guevara[1] , Mayerlyn Nathaly Ortega España[2] , Fernanda Elizabeth Cabrera Solarte[2] ,
Erick Fernando Collazos Chaves[3] , Arsenio Hidalgo Troya[4] , Andrés Darío Pantoja Bucheli[5] ,
Nelson Humberto Hurtado Gutiérrez[5] , Dolly Margot Revelo Romo[6]

[1]University of Nariño, Chemistry Department, Pasto, Colombia
[2]University of Nariño (Udenar), Chemistry Department, Pasto, Colombia
[3]University of Nariño (Udenar), Biology Department, Pasto, Colombia
[4]University of Nariño, Mathematics and Statistical Department, Pasto, Colombia
[5]University of Nariño, Electronics Department, Pasto, Colombia
[6]University of Nariño, Biology Department, Pasto, Colombia
Contact authors: cindycordoba74@gmail.com; mayerlyortega@udenar.edu.co; fercabre.sol@udenar.edu.co; fercollazos@udenar.edu.co; archi@udenar.edu.co;
pantoja@udenar.edu.co; nhurtado@udenar.edu.co; dmrevelor@udenar.edu.co
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ABSTRACT

The aim of this investigation was to compare the quality of coffee produced by dry fermentation using wet and honey-type methods in three farms located in southwestern Colombia through an ex post facto study. Specialty coffee was produced and studied according to a post-harvest protocol that utilized traditional practices. The study revealed that the quality of coffee is primarily affected by postharvest processing (57.6%) and the coffee farm (37.9%). The honey-like processing method employed on La Mina farm obtained the highest overall quality score, as determined by the Specialty Coffee Association (SCA). The variation in the cup quality occurred due to the variation in the different attributes. The overall impression, fragrance/aroma, body, and flavor were the primary contributors to the total score quality variance (34.9%), whereas acidity, aftertaste, and balance accounted for 26.1% of the total variance. Regarding the environmental, physicochemical, and microbiological parameters, the principal component analysis showed that the bottom temperature (BT), surface temperature (ST), middle temperature (MT), aerobic mesophilic bacteria (AMB), and °Bx accounted for 29.5% of the variance. Also, yeast, pH, LAB, and AAB accounted for 27.3% of the total variance. The highest correlation with the final cupping score was recorded for MT, ST, environmental temperature (ET), BT, Enterobacteria, and relative humidity (RH). Pre-fermentation was found to increase the microbial count, and longer dry fermentation durations of depulped coffee promoted the growth of beneficial microbial populations. The process revealed interesting relationships between temperature-AMB and degrees Brix (°Bx), and between yeast-lactic acid bacteria (LAB) and acetic acid bacteria (AAB).

Key words: *Coffea arabica*; Postharvest coffee processing; Wet and honey methods; Dry fermentation; Quality coffee.

1 INTRODUCTION

Coffee is an important product that drives the global economy. The International Coffee Organization (ICO) reported that around 70 countries were actively engaged in the production of Arabica coffee. Colombia is the third-largest coffee producer and exported 858,000 t of coffee in 2021 (International Coffee Organization - ICO, 2021). In 2022, Colombia exported 684,000 t of coffee, representing 10% of the country's total exports, which made coffee one of the main sources of foreign exchange income (Federación Nacional de Cafeteros de Colombia - FNC, 2023). Colombian coffee is popular for its quality, origin, production process, and tradition (Puerta, 2003). Several regions of Colombia compete in the international market for the production of distinct, high-quality varieties of coffee with denomination of origin (Superintendence of Industry and Commerce - SIC, 2005). Among these regions, the Department of Nariño is a key player in the production of Colombian coffee. In 2011, the title "CAFÉ DE NARIÑO" earned the status of a protected designation of origin, granted by the Superintendence of Industry and Commerce (SIC). The certification was attributed to the production of "washed Arabica coffee grown in coffee-producing regions of Nariño, which is characterized by its high acidity, medium body, sweet notes, and a clean, smooth, and very pronounced aroma"(SIC, 2011). These sensory or organoleptic attributes of Nariño coffee have contributed to its status as one of the finest varieties of coffee in the world. Coffee growers in the region have been awarded prizes, and they have earned prominent positions in various national and international competitions promoted by organizations that market specialty coffees (SIC, 2011).

To transform the coffee cherry into dry parchment coffee, the layers of the coffee fruit are removed using different methods, such as the dry or natural method, semi-dry or honey method, and wet or washed method (Pereira et al., 2019). In the dry processing method, coffee fruits are sun-dried or air-dried. In contrast, the wet processing method involves mechanical removal of the coffee skin and pulp, followed by microbial degradation (fermentation) of the mucilage layer, and finally, removal of water through sun-drying. In the semi-dry method, the coffee fruits are mechanically depulped before they are sun-dried. The beans obtained through any processing method must be dried till the final water content is around

10–12% (Pereira et al., 2019). In every processing method, the spontaneous fermentation of coffee beans is primarily promoted by the coffee microbiome (Zhang et al., 2019a). This microbiome exhibits significant variability, influenced by factors such as the cultivar and growing region, specific cultural practices, and postharvest processing methods (Vaughan et al., 2015; De Carvalho Neto et al., 2018b; Junqueira et al., 2019). During coffee fermentation, several factors, such as the coffee varieties, fermentation method, epiphytic microorganisms, temperature, pH, and acidity, influence the microbial diversity, metabolite formation, and consequently, the final coffee quality (Ribeiro et al., 2018). Coffee growers often apply traditional practices to conduct fermentation, which includes delaying pulping of coffee cherries (pre-fermentation) and fermenting depulped beans either by adding water in a tank (submerged or underwater fermentation strategy) or without water addition (dry fermentation or solid-state fermentation strategy). Pre-fermentation is a crucial stage that strongly affects coffee quality, although it is often poorly studied and is underestimated (Caixeta et al., 2013; Peñuela; Zapata; Durango, 2018; Zhang et al., 2019a). Similarly, the dry fermentation of coffee beans, commonly performed in countries like Colombia (Pacheco et al., 2018) and India (Velmourougane, 2013), needs to be further investigated.

The important physicochemical parameters (temperature, pH, and °Bx) and microbiological variables (microbial groups counts) that influence coffee fermentation need to be elucidated for comprehending and controlling the process to ensure the consistency and quality of the final cup. As far as the available scientific literature has been reviewed, there is a lack of comprehensive studies on these parameters. Using traditional plating methods, microbial groups have been isolated from fermenting coffee bean mass or detached microbial cells on fermenting cherries or beans. The findings have confirmed the presence of microorganisms and their variations throughout the process, particularly LAB and yeasts (Pereira et al., 2014, 2015). The decrease in the pH is a key characteristic of spontaneous coffee fermentation. The decrease in the pH is attributed to the microorganisms that use the mucilage as a carbon and nitrogen source, which leads to the production of large amounts of ethanol, lactic acid, and other microbial metabolites (Avallone et al., 2001; Pereira et al., 2014) The concentration of soluble solids (°Brix) is expected to decrease indicating sugar utilization in microbial metabolism (Lopez et al., 2015; Cruz-O'byrne; Piraneque-Gambasica; Aguirre-Forero, 2020; Aswathi et al., 2022; Pereira et al., 2022; Prakash et al., 2022). However, °Brix can also increase during coffee fermentation due to the action of microbial hydrolytic enzymes that break down carbohydrates in the mucilage (Junqueira et al., 2019). The temperature at which coffee is fermented might vary based on the environmental temperature and the method used (Aswathi et al., 2022). This may result in either a decrease (Junqueira et al., 2019), an increase (Córdoba; Guerrero, 2016; Prakash et al., 2022; Shankar et al., 2022), or adherence to the day-night cycle (Lopez et al., 2015; Zhang et al., 2019b). Microbial growth generates heat, and its enzymatic activity leads to an increase in temperature (Prakash et al., 2022). Temperature stabilization indicates the end of fermentation in the dry and semi-dry methods (Pereira et al., 2022). Additionally, the temperature of the coffee mass may vary depending on its position in the fermentation tank, such as the upper, middle, and bottom positions (Correa et al., 2014). Several studies have closely monitored these parameters during Arabica coffee underwater fermentation (Junqueira et al., 2019; Shankar et al., 2022), Arabica coffee dry fermentation (Lopez et al., 2015), and Canephora coffee dry fermentation (Prakash et al., 2022).

The relationship between these parameters and the cup quality was first investigated in the underwater fermentation of Colombian coffee (Cruz-O'byrne; Piraneque-Gambasica; Aguirre-Forero, 2020). Sensory evaluation, commonly referred to as the "cupping test", is used for classifying coffee throughout the world. This test serves as the principal methodology to assess the final cup quality (De Carvalho Neto et al., 2020; Prakash et al., 2022; Specialty Coffee Association - SCA, 2023). However, information on the comprehensive characterization of the dry fermentation of coffee in this region or the analysis of the cupping protocol is limited. The exceptional characteristics of Café de Nariño can also be attributed to the commonly used dry fermentation process, which is widely implemented in Colombia. Therefore, this ex post facto study aimed to compare the quality of coffee produced through dry fermentation utilizing wet and honey-type methods on three farms located in southwestern Colombia. The research findings could offer valuable insights and tools for comprehending and regulating the spontaneous fermentation of coffee.

2 MATERIAL AND METHODS

2.1 Coffee cherry collection, bean fermentation, drying, and sampling

Coffee cherries of *Coffea arabica* were harvested from July to August 2021, during the coffee harvest season, from three coffee farms in Buesaco, Nariño, Colombia: Loma Gorda (LG), La Mina (LM) and El Arrayán (EA) (Table 1 and Figure 1). These farms were chosen for their expertise in producing specialty coffees. The farms' coffee was grown in accordance with the Good Agricultural Practices Guides (FNC, 2007). Coffee processing was conducted following a protocol based on traditional practices where only healthy and mature coffee cherries were handpicked. Ripe cherries were immersed in water-filled tanks for sorting. The cherries were pre-fermented in woven polypropylene bags (50 kg) for 24 h and 48 h, and then, mixed with fresh cherries in

one batch at a ratio of 7:3. The hand-selected cherries (LG and LM) were mechanically depulped (Jotagallo No. 2¾ Marsella, Pereira, Colombia -LG, and Fimar No. 4½ and No. 2½ San Gil, Santander, Colombia -LM and EA). The depulped beans in LG and LM were then sorted using a rotary drum sieve (10 mm x 30 mm holes) and allowed to spontaneously ferment under dry conditions (dry fermentation) in a cement tank (LG: 1.70 m x 1.30 m x 0.70 m), in a cement tank covered with ceramic tile (LM: 1.41 m x 0.85 m x 0.59 m), and in a polyethylene tub (EA: 1.82 m x 1.19 m x 0.68 m) (Polinter 350, Bogotá, Colombia).

Table 1: Geographical, environmental, and processing data of coffee farms.

Farm	Loma Gorda (LG)	La Mina (LM)	El Arrayán (EA)
Rural area	Veracruz	Veracruz	Santa María
Longitude[a]	W: 077°09'30.9"	W: 077°09'23.6"	W: 077°07'13.9"
Latitude[a]	N:01°21'48.8"	N:01°21'16.7"	N:01°23'48.1"
Altitude (mamsl)	2120	1987	1957
First processing trial[b]	1108	2707	1108
Second processing trial[b]	1308	2907	1308
Environmental temperature (ET, °C) [c]	20.9 ±2.48 (1108) 20.4 ±2.41 (1308)	21.4 ±1.52 (2707) 21.0 ±0.60 (2907)	17.6 ±0.53 (1108) 17.0 ±4.65 (1308)
Relative humidity (RH, %) [c]	72.8 ±3.29 (1108) 79.9 ±5.65 (1108)	68.6 ±10.54 (2707) 61.9 ±4.53 (2907)	69.4 ±3.49 (1108) 98.9 ±1.96 (1308)
Coffee variety	Colombia (1108), Caturra, Castillo, Supremo (1308)	Castillo (2707) Castillo (2907)	Castillo, Colombia (1108); Castillo, Colombia and Caturra (1308)
Method	Wet	Honey-like	Wet

[a]Source: GPS measurement in site.
[b]Day and month information regarding the fermentation process during the 2021 harvest season: 1108 corresponds to August 11, 1308 corresponds to August 13, 2707 and 2907 corresponds to July 27 and 29.
[c]Average environmental temperature and relative humidity during coffee processing.

Figure 1: The location of the farms in the Buesaco municipality, Nariño, Colombia. Adapted from Google Earth.
Measurement of environmental and physicochemical parameters.

The depulped beans underwent fermentation for 10 h and 16 min (LG and EA) and 25 h and 20 min (LM). The coffee grower determines the duration of fermentation by feeling the friction between the coffee beans in their hands, which indicates the removal of the mucilage layer adhering to the beans. The fermented beans (except in the LM farm) were manually washed with clean water and then dried in a solar tunnel dryer until the moisture was reduced to 11–12%. Two processing trials (replicates) were conducted in the same week (Table 1). Samples, including 20 fresh cherries (FC) and 20 pre-fermented cherries (PC), were randomly collected from the batches set up for the day of processing. Additionally, at different times of fermentation, which were determined by initial test conducted on each farm, 10 g of coffee beans and mucilage were collected from different points of the upper, middle, and bottom sections of the tank and mixed evenly to represent the entire tank. (Elhalis; Cox; Zhao, 2020). All samples were collected in triplicate, placed aseptically in sterile plastic bags, and immediately transported to the laboratory in iceboxes for microbiological analyses.

2.2 Measurement of environmental and physicochemical parameters

The environmental temperature (ET, °C) and the relative humidity (RH, %) were continuously monitored using sensors (AM2301 sensor/module; Aosong Electronics Co., Ltd. Guangzhou, China) placed in the fermentation room. The pH and Brix degrees (°Bx) of the fermentation mass were measured using calibrated instruments, including a portable pen-type pH meter (Model 8681; AZ Instrument Corp., Taiwan, China), and a refractometer (Model RF15; Extech Instruments, Rotterdam, Netherlands). The temperature (°C) was monitored using sensors (DS18B20 digital temperature sensor; Maxim Integrated, California, USA) located at the surface (ST), middle (MT), and bottom (BT) of the fermenting mass. The data obtained from the sensors were automatically stored in a data logger system. All parameters were analyzed in quadruplicate at each sampling time throughout the fermentation process.

2.3 Quantification and Isolation of Microorganisms

Aliquots (10 g) of the coffee cherry or fermenting coffee bean samples were mixed with 90 mL of sterile peptone water (1 g L^{-1} bacteriological peptone from Oxoid, Basingstoke, England), and homogenized in an Orbital Shaker at 200 rpm for 20 min. These aliquots were used for decimal serial dilution (0.85% w/v NaCl; PanReac AppliChem, Barcelona, Spain) and plated in duplicate (Ribeiro et al., 2018; Zhang et al., 2019a; Elhalis; Cox; Zhao, 2020; Martins et al., 2020) Selective agar media and specific incubation conditions were used to target five microbial groups. Aerobic mesophilic bacteria (AMB) were quantified using plate count agar (PCA; HiMedia, Pennsylvania, USA) supplemented with 0.4 mg/mL nystatin (Sanfer, Bogota, Colombia) to inhibit fungal growth (Avallone et al., 2001; Ribeiro et al., 2018). Lactic acid bacteria (LAB) were cultured on MRS agar (Panreac Applichem, Barcelona, Spain) supplemented with nystatin (0.4 mg/mL) (Ribeiro et al., 2018). Yeasts and molds were cultivated on YGC medium (Condalab, Madrid, España) (Avallone et al., 2001). Acetic acid bacteria (AAB) were grown on modified deoxycholate-mannitol-sorbitol agar (mDMS) supplemented with nystatin (0.4 mg/mL) (Papalexandratou et al., 2013; Zhang et al., 2019b). Finally, Enterobacteria were targeted using violet-red-bile-glucose agar (VRBG) (Pronadisa, Madrid, Spain) (Zhang et al., 2019b). All agar plates were incubated aerobically at room temperature for 72 h to detect bacterial growth and up to five days to detect fungal growth, except for MRS, which was incubated anaerobically at room temperature in an AnaeroJar 2.5 L (Oxoid, Hampshire, United Kingdom) (Zhang et al., 2019a; Elhalis; Cox; Zhao, 2020). For each agar medium and sampling time an appropriate dilution containing 30 to 300 colonies was used to ensure accurate estimation (Renaloa, 2014). The counts (log CFU/g) were the means of duplicate analyses within a standard deviation of ± 0.6.

2.4 Coffee cup quality evaluation

Dry parchment coffee samples were collected ramdomly from each farm, packed in resealable plastic bags (1 Kg), and transported to the certified laboratory of Toldopamba SAS in La Piedra, Buesaco, Nariño. At the laboratory, the samples underwent hulling, roasting, and grinding to prepare the beverage for sensory assessment. The cupping protocol described by the SCA (2023) was followed by Toldopamba SAS to ensure accurate evaluation. A panel of four expert coffee tasters, all holding a Q-Grader Coffee Certificate, assessed the cup quality. The tasters evaluated various attributes of the coffee, including fragrance/aroma, flavor, aftertaste, acidity, body, balance, sweetness, clean cup, uniformity, and overall impression. Before the analysis, the panelists calibrated their palates at a tasting table. After tasting, the experts described the sensory characteristics and assigned a final quality score between 0 and 100 for each beverage (SCA, 2023).

2.5 Statistical analysis

To establish the consistency of the final scores for the attributes of coffee, provided by the four expert tasters, the intraclass correlation coefficient (ICC) was calculated. The ICC evaluates the agreement between two or more continuous measurements performed repeatedly in a series of subjects. It indicates reproducibility or reliability. A principal component analysis (PCA) was performed to identify the attributes that accounted for most of the common

variance in the scores given by the experts to the coffee cup. The attributes of sweetness, clean cup, and uniformity were excluded from these analyses, as they received similar scores from all four experts, which implied null variances. Normality and homoscedasticity were assumed, due to the size of the samples (40 per farm) and their balance. Additionally, PCA was conducted to determine the factors that explained most of the total variance of the environmental, physicochemical, and microbiological parameters. The analysis helped in establishing the relationship between these factors and the final score assigned to the coffee cup by the four experts. The differences among the final scores of the coffee cup were evaluated by conducting Student's t-tests and ANOVA, considering explanatory variables such as the method, farm, and the date of treatment. Additionally, to elucidate the effect of the first two variables on the variations in the scores, a nested ANOVA was performed. The wet method was used in the EA and LG farms, whereas the honey method was used in the LM farm.

3 RESULTS

3.1 Environmental and physicochemical characteristics during the dry fermentation of coffee

The environmental and physicochemical, characteristics recorded during the dry fermentation process of coffee at different durations of fermentation in each farm are shown in Table 2. During fermentation, the ambient temperature and relative humidity exhibited a typical inverse relationship in all processes. The recorded temperature and humidity ranges were 13.2–26 °C and 62.6–99.9% (EA) 15.0–16.6 °C and 68.1–83.8% (LG), and 19.7–23.5 °C and 54.9–83.8% (LM) (Table 2). The initial pH was around 4.3–5.2 for all processes and gradually decreased to 3.8–4.6 during fermentation. The major decrease in pH occurred after 4–5 h (EA and LG) and 11 h (LM). Soluble solids showed a range of 16.5–19.0 °Bx (EA), 14.8–16.6 °Bx (LG), and 14.6–18.0 °Bx (LM). The values of °Bx increased and decreased in all processes during fermentation. The middle and bottom areas of the fermentation mass showed similar temperature changes, with an average increase of 2.27 °C (EA 1108), 5.69 °C (EA 1308), 4.74 °C (LG 1108), 5.44 °C (LG 1308), 1.76 °C (LM 2707), and 4.35 °C (LM 2907), relative to ET (Table 2). The upper area of the fermentation mass increased (on average) by 2.18 °C (EA 1308), 2.98 °C (LG 1108), 5.55 °C (LG 1308), and 7.11 °C (LM 2907), but it decreased by 0.96 °C (EA 1108) and 1.93 °C (LM 2707), relative to ET. In general, the highest temperatures were recorded in the middle of the tank.

The populations of AMB, LAB, AAB, Enterobacteria, and yeast observed during fermentation at the three farms are shown in Figure 2 and Suplementary Figure S1. Microbial counts on the surfaces of the fresh cherries (FC) ranged from log 2.0 to log 6.5 (CFU/g), while for the pre-fermented cherries (PC), the microbial counts ranged from log 3.3 to log 7.2 log (CFU/g) (Supplementary Figure S1). The pre-fermented cherries had the highest microbial counts (Figure 2 and Supplementary Figure S1). The average microbial count for the cherries revealed the following: lower counts were found on mMDS and MRS [log 3.0 and log 3.9 (CFU/g)], which indicated that the presence of presumptive AAB and LAB in the fresh cherries was relatively low. After pre-fermentation, the average lower count on mMDS was log 4.8 (CFU/g). The microbial populations of the cherries increased after pre-fermentation in the following order: LAB, AAB, AMB, Enterobacteria, and yeast.

The targeted microbial groups on the fermenting depulped coffee beans showed variations in growth patterns across all fermentation processes, including differences between the first and second processing trials (Figure 2 and Supplementary Figure S1). It found that AMB was the most prevalent microbial group in all fermentation processes, with counts ranging from log 6.7 to log 9.5 (CFU/g) (Supplementary Figure S1). They were followed by presumptive LAB with counts of log 5.7–8.5 (CFU/g), and presumptive yeast with counts of log 5.5–8.1 (CFU/g). No filamentous fungi were detected in any process. Compared to other microbial groups, the counts of AAB (log 4.6-7.7 CFU/g) and Enterobacteria (log 3.9-7.2 CFU/g) were slightly lower. Nevertheless, at the end of the fermentation process, Enterobacteria had a higher count than the other microorganisms at the Loma Gorda farm (1108). The first processing trial had higher counts than the second processing trial in all farms.

3.2 Sensory evaluation of the coffee drink

The average scores given by the four tasters for each cup attribute, including fragrance/aroma, flavor, aftertaste, acidity, body, balance, overall impression, uniformity, clean cup, and sweetness, are shown in Figure 3 (A-F).

Attributes such as uniformity, clean cup, and sweetness received a perfect score of 10 across all three farms (Figure 3). Among the attributes that received higher scores, fragrance/aroma, aftertaste, and overall impression were prominent in the LM farm (Figures 3 E and F), whereas the flavor of coffee was strong in the LG farm (Figure 3 C). Acidity (Figures 3 D-F), body (Figures 3 D and E), and balance (Figures 3 C and F) were prominent in the LG and LM farms. The final cupping scores for the three farms were as follows: 83.13 and 83.44 for EA, 84.50 and 83.94 for LG, and 85.38 and 84.94 for LM.

Table 2: Environmental and physicochemical characteristics of the dry fermentation of coffee in the three farms of the municipality of Buesaco.

Farm	Fermentation time (h)	Physicochemical parameters of the fermentation mass			Environmental parameters	
		pH	Brix (°Bx)	Temperature (°C)a	Environmental temperature (ET, °C)	Relative humidity (RH, %)
El Arrayán (EA) (1108)	0	4.43 ±0.05	17.7 ±0.34	18.5 ±1.20	18.6 ±0.26	62.6 ±0.53
	2.25	4.33 ±0.15	17.6 ±0.43	19.0 ±2.04	17.1 ±0.21	70.2 ±0.99
	4.25	3.75 ±0.06	17.8 ±1.07	18.9 ±1.80	17.2 ±0.47	72.5 ±4.54
	6.25	4.08 ±0.10	19.0 ±1.38	19.0 ±1.96	17.6 ±0.05	71.2 ±1.12
	9.33	3.98 ±0.10	17.3 ±0.77	18.6 ±2.39	17.6 ±0.29	70.4 ±4.87
El Arrayán (EA) (1308)	0	5.13 ±0.10	17.8 ±1.0	20.8 ±0.13	26.0 ±0.49	95.0 ±3.16
	3	4.93 ±0.10	18.0 ±0.8	19.8 ±1.27	16.7 ±0.14	99.9 ±0.00
	6	4.68 ±0.13	16.5 ±0.6	19.2 ±2.06	14.7 ±0.08	99.9 ±0.00
	9	4.63 ±0.15	16.8 ±1.3	19.0 ±2.41	14.3 ±0.13	99.9 ±0.00
	11	4.63 ±0.17	16.5 ±0.6	19.0 ±2.52	13.2 ±0.22	99.9 ±0.10
Loma Gorda (LG) (1108)	0	5.20 ±0.00	16.5 ±0.58	23.6 ±0.90	25.5 ±0.00	68.1 ±0.00
	3	5.23 ±0.10	14.8 ±1.50	23.6 ±0.72	21.6 ±0.00	69.8 ±0.35
	6	4.93 ±0.17	14.8 ±2.06	24.0 ±0.88	19.3 ±0.10	74.3 ±0.59
	9	4.58 ±0.10	15.0 ±0.82	24.2 ±1.08	19.2 ±0.13	75.3 ±0.30
	10.48	4.28 ±0.10	15.3 ±1.50	24.2 ±1.11	19.0 ±0.00	76.6 ±0.30
Loma Gorda (LG) (1308)	0	4.85 ±0.17	15.0 ±0.00	24.1 ±0.43	24.5 ±0.92	68.7 ±0.57
	2.67	4.60 ±0.16	16.0 ±0.00	24.2 ±0.42	21.6 ±0.00	83.3 ±0.12
	4.67	4.38 ±0.25	16.6 ±0.12	24.4 ±0.42	19.6 ±0.05	81.8 ±0.45
	6.67	4.33 ±0.05	16.0 ±0.08	24.8 ±0.40	18.9 ±0.19	81.8 ±1.10
	10.27	4.13 ±0.10	15.5 ±0.00	25.7 ±0.37	17.6 ±0.05	83.8 ±0.25
La Mina (LG) (2707)	0	4.38 ±0.15	16.9 ±0.63	21.1 ±0.70	22.7 ±0.21	57.8 ±0.42
	6	4.40 ±0.14	17.7 ±0.43	21.3 ±0.94	21.2 ±0.13	80.9 ±0.70
	12	3.98 ±0.05	18.0 ±0.00	21.1 ±1.99	19.8 ±0.60	79.3 ±0.88
	18	3.70 ±0.14	16.9 ±0.25	21.6 ±2.03	19.7 ±0.19	69.7 ±1.89
	25.67	3.78 ±0.15	15.6 ±1.11	22.7 ±1.49	23.5 ±0.17	55.5 ±0.54
La Mina (LG) (2907)	0	4.30 ±0.00	16.4 ±0.47	25.7 ±0.86	20.5 ±0.06	67.4 ±0.68
	6	4.03 ±0.10	17.7 ±0.35	26.0 ±0.94	21.2 ±0.10	57.0 ±0.21
	12	3.98 ±0.05	16.5 ±0.46	26.4 ±1.33	20.9 ±0.08	66.1 ±1.53
	18	3.95 ±0.06	15.3 ±0.81	26.7 ±1.69	20.3 ±0.17	62.5 ±0.52
	25	3.80 ±0.08	15.0 ±0.00	27.2 ±2.23	22.0 ±0.08	±0.10

aAverage temperature of the fermentation mass.
Microbial group count dynamics during coffee fermentation.

The measurement of cup quality by tasters was reliable. The ICC was 0.499, and the 95% confidence interval ranged from 0.1063 to 0.8849. This value indicated adequate reliability in the measurement of cup quality according to Fleiss's scale for assessing the ICC (Fleiss, 1986). The variables Overall impression, fragrance/aroma, body, and flavor attributes, contribute with the greatest variability in the coffee cup quality scores, component 1 that contributes 34.9% of the total variation of said scores. The second component consisted of the attributes acidity, aftertaste, and balance, which together explained 26.1% of the variance. The total variance of all measured attributes was 60.9% (Table 3).
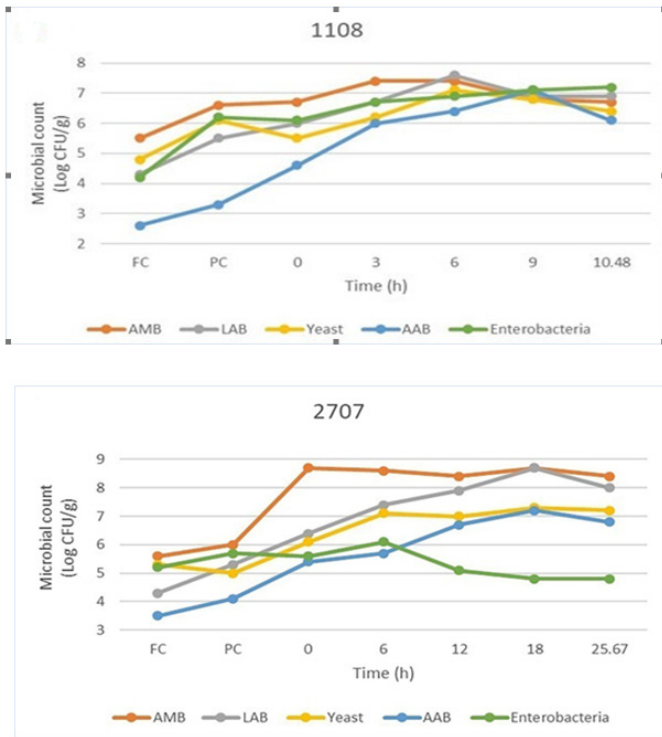
Figure 2: The microbial counts of cherries and fermenting beans of Arabica coffee, recorded in the wet and honey-like processes that obtained the highest cupping score, are shown for; Loma Gorda: 1108 and La Mina: 2707.

3.3 Effects of the evaluated variables on the final score of coffee cup

The scores of the coffee cups determined by the method and farm variables are shown in Table 4. The honey-like method showed higher scores than the wet method (p < 0.001). The coffee cup from the LM farm, where coffee was grown using the honey-like method, showed higher average scores than the other two farms, where coffee was grown using the wet method. The results of the Bonferroni multiple comparison test showed significant differences in the scores among the three farms, which indicated that the farm is a key factor associated with the scores. The wet method was used in the EA and LG farms, whereas the honey-like method was used in the LM farm. The differences between the dates of treatment were not significant. A nested ANOVA was performed to determine the percentage contribution of the method and farm variables to the variation in the coffee cup scores. The results showed that the method contributed 57.6% to the variability, whereas the farm contributed 37.9% to the variability (Table 4); the remaining 4.5% was attributed to error.

The PCA of the environmental, physicochemical and microbiological variables allowed us to establish that the first component was made up of the variables BT, ST, MT, AMB and °B which contributed with approximately 29.5% of the total

variation of said set of variables. The second component consisted of the variables yeast, pH, LAB, and AAB, which contributed 27.3% to the overall variability. The parameters RH, ET, and Enterobacteria constituted the third component and contributed to 19.4% of the total variance. Together, these three components accounted for 76.3% of the variability in the data (Table 5).
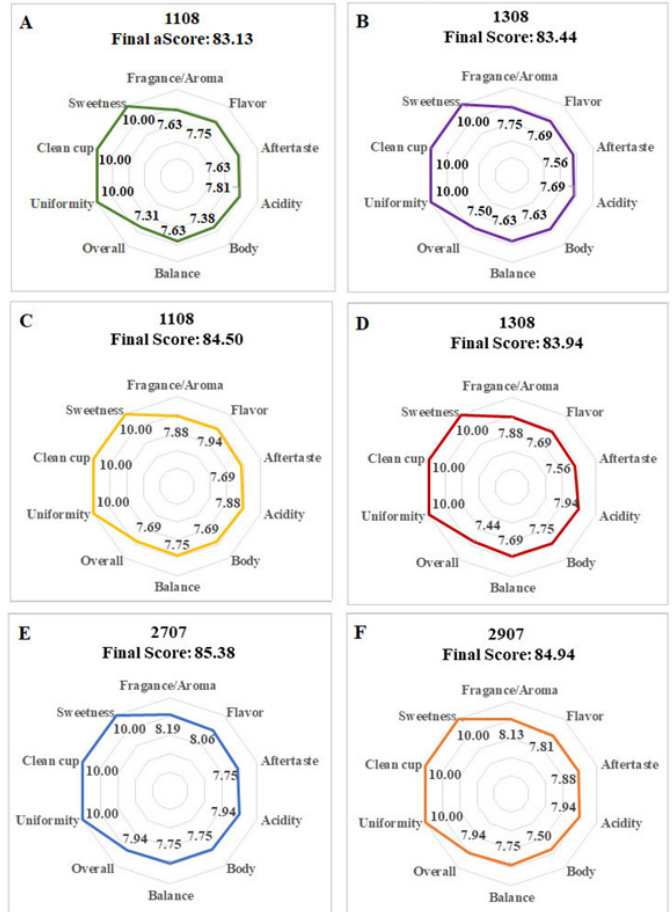

Figure 3: Sensory profiling analysis and Final Score of *Coffea arabica* from three farms. El Arrayán A) 1108 and B) 1308; Loma Gorda: C) 1108 and D) 1308; La Mina: E) 2707 and F) 2907.

Table 3: Component loadings of the scores for the attributes of the cups of coffee.

	Component	
	1	2
Overall	0.830	
Fragrance/Aroma	0.773	
Body	0.729	
Flavor	0.609	
Acidity		0.828
Aftertaste		0.692
Balance		0.562

Note. 'varimax' rotation was used.

Table 4: Statistics of coffee cup scores based on the method and farm.

Variables		n	mean	s.d.	P value	Comp Var	%
Method	wet	80	83.75	0.52	<0.001	0.663	57.6%
	Honey-like	40	85.16	0.22			
Farm	EA	40	83.29	0.16	<0.001	0.436	37.9%
	LG	40	84.22	0.28			
	LM	40	85.16	0.22			
Error						0.052	4.5%
Total						1.151	100.0%

Table 5: Component loadings of environmental, physicochemical, and microbiological parameters.

	Component		
	1	2	3
BT (°C)	0.908		
ST (°C)	0.871		
MT(°C)	0.866		
AMB	-0.739		
°Bx	-0.722		
Yeast		0.896	
pH		-0.843	
LAB		0.810	
AAB		0.776	
RH (%)			-0.841
ET (°C)			0.827
Enterobacteria			-0.564

Note: 'varimax' rotation was used.

Most of the environmental, physicochemical, and microbiological parameters showed significant correlation with the final scores (Table 6). The highest correlations were recorded for MT, ST, ET, BT, Enterobacteria, and RH, whereas AMB, yeast LAB, and AAB showed no significant correlations.

4 DISCUSSION

At each of the farms, a post-harvest coffee processing protocol was implemented utilizing traditional practices to produce specialty coffee. Therefore, according to the total score for classifying the quality described by the SCA (2023), the coffee samples were categorized as Excellent – Specialty (LM 2707) and Very Good – Specialty (all other farms). The evaluation of cup quality was reliable, as the observed variability mainly occurred due to the differences between the coffee cups rather than variations in the measurement methods used by the tasters. The tasting table, where panelists calibrated their palates,

played an important role in ensuring that the evaluations were consistent and accurate. The attributes grouped into the first and second components accounted for most of the variance in the cup quality, as they determined the unique flavor and aroma qualities, which are the characteristics of specialty coffee. The coffee cultivated on the La Mina farm had the highest scores for some of the most important coffee attributes, such as fragrance/aroma, aftertaste, and the overall impression. The attributes excluded from the analysis in all three farms might represent the fundamental characteristics of Nariño coffee quality. The results of the test indicated that only certain attributes were necessary to determine the perception of quality.

The statistical analysis revealed that the honey-like method produced better scores, followed by the wet method. Nevertheless, improvements are required in the selection processes of cherries and depulped beans on the EA farm to improve the cup quality of the wet method. Some studies have found that processing methods strongly influence the microbial community structures and, consequently, the final composition of green coffee beans (De Bruyn et al., 2017; Zhang et al.,

Table 6: Correlation coefficients of the score with the environmental, physicochemical, and microbiological parameters.

Variable	Correlation	Valor p
ET (°C)	0.509	< .001
RH (%)	-0.495	< .001
pH	-0.270	0.003
°Bx	-0.276	0.002
BT (°C)	0.454	< .001
MT (°C)	0.616	< .001
ST (°C)	0.538	< .001
AMB	0.151	0.099
Yeast	0.144	0.116
LAB	-0.048	0.602
AAB	0.010	0.911
Enterobacteria	-0.607	< .001

2019b). On the other hand, the processes that utilized a single coffee variety yielded the highest total scores for cup quality. It is recommended that future studies compare a single coffee variety within the same farm for further evaluation.

Furthermore, variations in cup quality may be due to the subjective nature of determining the end-point of the fermentation process, resulting in coffee growers fermenting for different durations. The coffee grown on the LM farm had the highest total score, with a fermentation time almost twice as long as those grown on the LG and EA farms. The research conducted by Zhang et al. (2019a, 2019b) found that the duration of underwater fermentation had the greatest effect on green coffee bean composition and cup quality due to changes in microbial and metabolite profiles. Additionally, the quality of the fermented coffee beans may have been further enhanced during the drying phase on LM farm due to the continued microbial degradation of the residual mucilage, which was not washed off but left in the open.

The variation in the range of ET and RH among the three farms was low, indicating that dry fermentation was conducted under similar environmental parameters. These two variables, along with the variable Enterobacteria, were grouped to form a third component, which contributed slightly to the variation across dates. The first and second components (BT, ST, MT, AMB, °Bx) and (yeast, LAB, AAB, and pH), respectively, contributed significantly to the total variance of all measured parameters. The microbial relations could result in the production of metabolites that positively affect the sensory attributes of coffee (Junqueira et al., 2019, Cruz-O'byrne; Piraneque-Gambasica; Aguirre-Forero, 2021). Strong direct correlations between the relative abundance of Lactobacillus, fermentation duration time, and fermentation type have recently been reported (Peñuela; Velasquez; Angel, 2023). The final statistical analysis revealed a significant positive correlation between the total score and both the environmental temperature (ET) and the fermentation mass temperatures (BT, MT, ST). Enterobacteria exhibited a statistically significant negative correlation with the total score.

The fermentation mass temperature increased relative to the ET, specifically in the middle of the tank. In different varieties of Colombian Arabica coffee fermented under dry conditions, the highest radial gradient was found in the surface and middle planes, and the lowest was recorded in the floor plane (Correa et al., 2014). The increase in temperature was attributed to the degradation process and exothermic reactions during microbial growth (Velmourougane, 2013; Prakash et al., 2022). This increase may have been primarily influenced by the metabolism of AMB, which recorded the highest counts in all of the trials. The fluctuations in °Bx observed during the dry fermentation process of coffee may be attributed to microbial metabolism, which promotes the breakdown of complex carbohydrates and increases glucose

and fructose levels for later consumption (Junqueria et al., 2019).

Although various pH values were noted at the end of fermentation, the decline in pH can be utilized as a determinant for coffee growers to determine when the process is complete. Microbial metabolism is also related to acidification due to the production of metabolites, such as organic acids, which can lead to a decrease in pH and facilitate the breakdown of the mucilage polysaccharide network (Avallone et al., 2002). Lactic acid contributes to the decrease in the pH and is correlated with the growth of LAB (De Carvalho Neto et al., 2018a, 2020; Junqueira et al., 2019). In this study presumptive LAB were also found to be a prevalent microbial group in all fermentations. The metabolic activity of LAB decreases the pH, which results in optimal conditions for the growth of yeast (Nasanit; Satayawut, 2015). In this study, yeasts were found to be another prevalent microbial group. They play a crucial role in coffee fermentation as they generate various aroma-influencing molecules through central carbon and nitrogen metabolism (Pereira et al., 2019). Their growth and activity can also inhibit the proliferation of undesirable microorganisms, such as filamentous fungi, and the production of undesirable metabolites (Elhalis; Cox; Zhao, 2020). These effects might be responsible for the absence of filamentous fungi in the dry fermentation process performed in this study. In contrast, fungal populations were generally higher during the underwater fermentation process than the bacterial and yeast populations in the specialty coffee produced in northern Colombia (Cruz-O'byrne; Piraneque-Gambasica; Aguirre-Forero, 2020).

AAB and Enterobacteria appeared to be less competitive in the dry fermentation environment. The exact role of AAB in fermentation remains unclear (Elhalis; Cox; Zhao, 2020); however, it was found that AAB can interact synergistically with other microbial populations, such as LAB, and promote the production of metabolites that positively affect the sensory attributes of coffee (Cruz-O'byrne; Piraneque-Gambasica; Aguirre-Forero, 2021). Enterobacteria is mainly associated with human contact and the formation of off-flavor metabolites (Junqueira et al., 2019).

This study presents the first comprehensive quantification of all five of the aforementioned microbial groups in the dry fermentation of coffee. AMB, LAB, and yeast were the most prevalent microbial groups, whereas AAB and Enterobacteria were less abundant. These findings matched the results reported in previous studies on underwater fermentation (Avallone et al., 2001; Zhang et al., 2019b). The microbial populations showed variations during the fermentation of the depulped beans and in each processing trial. Although both trials were conducted in the same week, several factors, such as the recent cleaning of the processing area for the second trial and a greater increase in the middle and bottom temperatures related to the fermentation mass in

the second trial, probably contributed to the lower microbial counts recorded.

A substantial increase in populations, especially of LAB, was observed after the pre-fermentation process in the present study. As a result, LAB could have potentially colonized the fermentation mass from the surface of the cherries during the dry fermentation process. In the period between harvest and depulping, similar results were obtained for the Typica variety (Zhang et al., 2019a). This increase in microbial communities might be attributed to the release of carbohydrate-rich juices from the coffee cherries due to the mechanical pressure exerted during storage (Zhang et al., 2019b). LAB communities and their metabolites can act as a shield, protecting the ecosystem from these microbes and allowing coffee beans to display their extensive endogenous metabolism (Zhang et al., 2019a). This might have occurred during the fermentation process in this study, particularly in the LM farm, where the counts of Enterobacteria were the lowest. Such factors might have contributed to the production of high-quality specialty coffee.

5 CONCLUSIONS

An ex post facto study was conducted using a sample design to compare the quality of coffee produced by dry fermentation through wet and honey-type methods in three farms located in Southwestern Colombia with variations in their environmental and physicochemical characteristics. Sensory characteristics demonstrating unique qualities specific to the geographic region of Nariño coffee production have been uncovered. The honey method applied to the beans grown on the La Mina farm produced the highest quality coffee, followed by the wet method applied on the Loma Gorda farm. All the physicochemical and microbiological parameters evaluated in the dry fermentation of coffee, except Enterobacteria and environmental parameters, had greater variability in the process. The parameters most positively related to coffee quality were the ambient temperature and the temperature of the fermenting mass. The temperature of the fermentation mass similarly affected the microbial counts, which, in turn, affected the quality of the cup. The microbial counts during dry fermentation of depulped coffee were influenced by the post-harvest processing method, prefermentation, and duration of fermentation, which affect cup quality. Among the microbial groups detected during dry fermentation, AMB, LAB and yeasts had the highest prevalence. By slightly modifying the method and fermentation time, Nariño coffee growers who produce specialty coffee might be able to considerably increase and consistently reproduce the superior taste and quality of coffee. It is essential to regulate specific fermentation parameters as

they have the potential to impact the sensory features and microbial environment of coffee.

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7 AUTHORS' CONTRIBUTIONS

Conceptual idea: Revelo, D.; Pantoja, A.; Hurtado, N.; Methodology design: Revelo, D.; Hurtado, N.; Pantoja, A.; Data collection: Revelo, D.; Córdoba, C.; Ortega, M.; Cabrera, F.; Collazos, E.; Data analysis and interpretation: Hidalgo, A.; Revelo, D.; Hurtado, N.; and Writing and editing: Córdoba, C.; Ortega, M.; Cabrera, F.; Collazos, E.; Hidalgo, A.; Pantoja, A.; Hurtado, N.; Revelo, D.

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