

Effect of *Pichia kudriavzevii* addition on coffee fermentation during natural and washed process methods

Angélica Piedad Sandoval Aldana¹ , Yerly Patricia Cardona Montoya² , Edwar Andrey Leon Linares³ ,
José Daniel Guarnizo Jiménez^{3*} 

¹Universidad del Tolima - Departamento de producción y sanidad vegetal. Ibagué, Tolima, Colombia

²Gobernación del Tolima - Secretaría de Planeación Ministerio de Ciencia, tecnología e innovación. Supervisión proyectos Regalias, Ibagué, Tolima, Colombia

³Universidad del Tolima - Facultad de ingeniería agronómica Laboratorio LABCAFE. Ibagué, Tolima, Colombia

*Corresponding author: jdgurnizo@ut.edu.co

Editor: Samuel Pereira de Carvalho 

Received in August 27, 2025 and approved in April 13, 2026

ABSTRACT

Fermentation is a metabolic process mediated by microorganisms that plays a critical role in coffee processing by promoting mucilage degradation and converting sugars such as glucose, fructose, and non-reducing sugars into simpler metabolites, including organic acids, alcohols, and esters, which contribute to the development of volatile compounds expressed during roasting, such as 2-methylpyrazine and furfurylthiol. In this research, the influence of *Pichia kudriavzevii* yeast on the fermentation of Castillo® variety under field environmental conditions (48.00, 72.00 h fermentation; 17.00°C-42.00°C drying temperature; 9.50-12.50% final moisture content) were evaluated. There were significant differences in the consumption of reducing sugars in relation to fermentation time (48.00 h and 72.00 h), processing type (Natural process, Washed process), and fermentation method (*Spontaneous fermentation* and *Inoculated* with *P. kudriavzevii* yeast). During the SCA sensory evaluation, attributes such as chocolate and vanilla fragrances/aromas were characterized in the *Traditional Washed* process (Colombian traditional postharvest method consisting of sorting cherries, depulping, fermenting, washing and drying) with spontaneous fermentation. By contrast, washed inoculated treatment obtained fruity notes such as blueberries, oranges, pineapple, and strawberries. SCA evaluation for *Natural* process with spontaneous fermentation showed notes such as winey, chocolate, caramelized, while *Natural* inoculated treatment was characterized by black tea, brown sugar and blueberry. On the other hand, the processing type presented significant differences, demonstrating influence on the sensory quality of coffee, where the highest score obtained was 86.13, corresponding to the *Natural* control of 48.00 h.

Key words: *Coffea arabica*; glucose; natural process; *pichia kudriavzevii*; starter culture, sensorial attribute.

1 INTRODUCTION

Coffee is a highly relevant raw material that has been preserved over the years, becoming the second most traded commodity worldwide, only behind crude oil (Haile & Kang, 2019a). Among the 81 coffee-producing countries, Colombia is recognized for its high sensory quality and is ranked third in the most producer countries based on volume 12.56 million of 60,00 kg of green coffee bags (International Coffee Organization - ICO, 2021). The development of differentiated physicochemical and sensory characteristics in coffee plants is derived from the genotype-by-environment ($G \times E$) interaction, understood as a dynamic relationship in which the genetic makeup of the plant (genotype) expresses distinct traits depending on the environmental conditions in which it grows (Santos et al., 2018). For *coffea arabica* L., the most significant environmental factors influencing coffee quality are altitude (1,000-2,000 m.a.s.l.), latitude (near equatorial line), temperature (aprox. 20°C), and rainfall (1,460-1,700 mm), which directly impact the chemical composition of the beans and the sensory attributes of coffee, even between *Coffea arabica* and *Coffea canephora* species (Figueiredo et al., 2015).

In relation to the post-harvest process, it has been proven that this influences the chemical and sensory composition of the bean. This makes it essential to maintain

and standardize the quality of the final product (Bressani et al., 2021b). The conventional approach to coffee processing has been through the wet or washed method which is characterized by the removal of both pulp and mucilage (Cruz-O'Byrne et al., 2021). Natural drying process, which involves exposing coffee cherries to sunlight until the desired moisture content is reached, is characterized by imparting body to the beverage, smoothness, sweetness, and cup complexity. This method allows for spontaneous fermentation due to the presence of mucilage and pulp surrounding the coffee bean (Hameed et al., 2018).

However, in recent years, there has been a shift towards alternative methods due to the environmental impact of traditional practices such as improved washed process, and natural drying process represents one of these alternatives. In each of these processing methods, it is imperative to consider the natural microbiota present in the coffee cherry, because different positive impacts have been identified on the quality of the final product. These microorganisms possess the capacity to spontaneously initiate the fermentation process, which functions to convert the outer layers of the bean (Elhalis, Cox & Zhao, 2023).

Fermentation is a critical process in the characterization of coffee's sensory components, in which the primary objective is the degradation of mucilage. This metabolic process is

facilitated by the action of yeasts and various bacteria, which transform organic chemical compounds, such as sugar, into simpler substances, including ethanol, organic acids, and other compounds that enhance the sensory qualities of coffee (Peñuela-Martínez et al., 2018; Puerta, 2010). Due to the diversity of microbial genera present in coffee fermentation, it is ideal to carry it out in a controlled manner to guarantee standardized quality. One possible alternative is to use microorganisms as starter cultures, which have been associated with greater control over pathogenic microorganisms and its positive effects on coffee sensory profile. This, in turn, has been shown to provide stability and reliability of fermentation yield, as well as to provide the beverage with differentiating sensory characteristics of all kinds (Bressani et al., 2021b; Silva Vale et al., 2021; Carvalho Neto et al., 2017; Souza et al., 2017; Pregolini et al., 2021).

According to several authors, yeasts are regarded as optimal starter cultures due to their fermentative capacity, high pectinolytic activity, and tolerance to stress during fermentation. Moreover, they have been demonstrated to produce flavoring compounds and to inhibit the growth of mycotoxin-producing filamentous fungi. Based on these characteristics, *P. kudriazevii* (commonly known as *Candida krusei*) has been identified as a promising starter in coffee fermentation (Bressani et al., 2021a; Elhalis et al., 2021; Haile & Kang, 2019b; Shankar et al., 2022).

In the present research, an analysis and comparison of two coffee processing methods (dry or natural, and wet or traditional washed) was carried out, and the effect generated by fermentation with a starter culture was observed. In this study, the fundamental role played by *P. kudriazevii* in comparison with spontaneous fermentation in the postharvest process among two types of coffee processing methods was investigated. It is anticipated that the findings of this study will serve as a foundational framework for subsequent research, thereby facilitating the optimal development of coffee growers.

2 MATERIAL AND METHODS

2.1 Raw Material

The raw material used was Castillo® variety (arabica var. coffee), which was obtained from the La Noreña farm, Puerto Perú sidewalk, municipality of Ibagué, Colombia, located at an altitude between 1,550 and 1,620 m.a.s.l.. The cherries were harvested and classified at their optimum state of maturity.

2.2 Selection of Microorganism

The strain *P. kudriazevii* was obtained from the strain preserved in the postharvest and quality control laboratory

at Universidad del Tolima in Ibagué, Colombia, isolated from spontaneous fermentation processes of cocoa in the municipality of Ibagué, (Tolima) of the cocoa variety CCN-51 (Criollo Nuñez et al., 2023). The strain was concentrated on a concentration of 1×10^7 CFU/mL using a diluent solution for subsequent use.

2.3 Experiment Design

The factors to be studied were: two (2) fermentation times (48.00 hours and 72.00 hours), 2 types of fermentation (spontaneous or control, and inoculated with *P. kudriazevii*), and two (2) types of processing (Natural and Traditional Washed) with two independent micro-lots of 15.00 kilograms per treatment (processed in duplicate).

2.4 Coffee Processing

The coffee cherries (15.00 kg per bag) were washed and disinfected with salt used at a concentration of 2.00% w/v per liter of water and white vinegar at 1.00% v/v, leaving the mass of coffee cherries under immersion for 3.00 to 5.00 minutes to reduce the initial microbial load. Then, they were assigned to the experimental treatments. All fermentations were carried out in GrainPro® Zipper RZ-15 bags in the same room at a defined temperature (22.00 °C) and relative humidity (85.00 %) for the defined fermentation times.

Whole cherries with intact outer layers (skin, pulp, and mucilage) were placed directly into GrainPro® bags for fermentation in the natural treatment. For traditional washed treatment, the cherries were depulped, and the resulting parchment coffee with fermented mucilage was placed in GrainPro® bags and fermented under the same conditions. During fermentation, a liquid leachate accumulated around the coffee parchment in both treatments. After the target fermentation time, the traditional washed coffee was rinsed with potable water to remove the leachate and residual mucilage before being transferred to the drying stage.

The leachate generated from the fermentation of natural and traditional washed treatments was collected and transported to the post-harvest and quality control laboratory at the University of Tolima in Ibagué, Colombia. The laboratory is in the Andean coffee-growing region at approximately 4.438° N and 75.200° W. There, we monitored and analyzed its physicochemical characteristics (pH, titratable acidity, total sugars, and reducing sugars). Titratable acidity was determined by titration and expressed as acetic acid equivalents. After sample collection and completion of fermentation, both treatments were dried on raised beds until the coffee beans reached a moisture content between 9.50% and 12.50%. Figure 1 summarizes the overall workflow of the post-harvest processing.

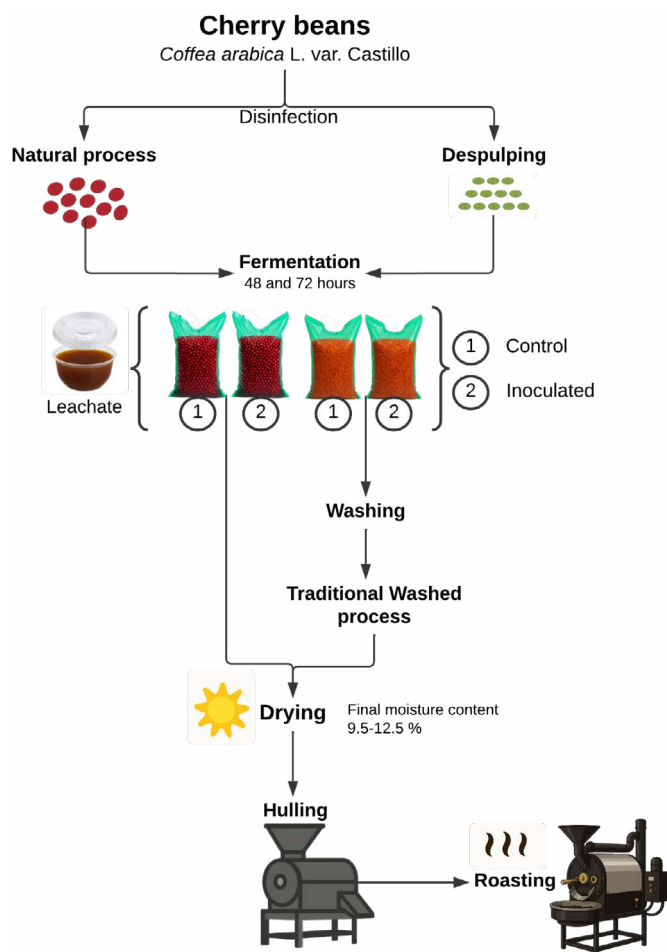


Figure 1: Schematic representation of the experimental workflow (Self-Authored).

2.5 Total Sugars

Total sugars concentration was quantified using the anthrone method (0.20% m/v anthrone), as described by López-Legarda et al. (2017). A calibration curve was established with glucose standard solutions ranging from 80.00 to 800.00 milligrams per liter (mg/L), utilizing distilled water as the blank. The experiment was conducted in Eppendorf tubes. Initially, 50.00 μL of the leachate sample was added to each tube, which was then placed in an ice bath for 10.00 minutes. Following this, 100.00 μL of anthrone (10H-antracen-9-ona) was carefully added along the walls of the tubes, and they were returned to the ice bath for an additional 5.00 minutes. Subsequently, the contents of the tubes were homogenized using a vortex mixer (DLAB Scientific, China, 2016). The tubes were then transferred to a water bath (Mettler, Germany) set at 100.00 $^{\circ}\text{C}$ for 10.00 minutes. After removal from the water bath, they were placed in an ice-water bath (0.00 $^{\circ}\text{C}$) for an additional 10.00 minutes. Following this, 100.00 μL of the solution was transferred from each tube to a 96-well microplate. All procedures were

performed in triplicate, and the resulting optical density was measured at 630.00 nanometers using a Multiskan SkyHigh spectrophotometer (Thermo Scientific, Singapore). Total sugars in the fermentation leachate were quantified for all treatments and fermentation times evaluated, and the results were expressed as g/L glucose equivalents.

2.6 Reducing Sugars

The quantification was performed using the Miller 3,5-dinitrosalicylic acid (DNS) method, with specific modifications. Glucose was utilized as the standard solution in this study (Ávila Núñez et al., 2012). A calibration curve was established for glucose concentrations ranging from 625.00 to 5.000 milligrams per liter (mg/L), alongside a blank sample consisting of distilled water. The analysis was conducted in Eppendorf tubes, to which 50.00 μL of the sample and 50.00 μL of DNS reagent were added. Subsequently, the tubes were placed in a water bath maintained at 90.00 $^{\circ}\text{C}$ for a period of five minutes. Consecutive to the heating period, the tubes were permitted to cool. Thereafter, 500.00 μL of distilled water was added to each. Subsequently, 100.00 μL of the resulting solution from each tube was transferred to a 96-well microplate. The experiments were conducted in triplicate, and the optical density was measured at 540.00 nanometers using the Multiskan SkyHigh spectrophotometer (Thermo Scientific, Singapore). The concentration of reducing sugars in the leachates of each sample was determined from the interpolated absorbance values, considering a sample dilution factor of 10^2 . The reduction in sugars was expressed in grams per liter of glucose equivalents.

2.7 Non-Reducing Sugars

The quantification of non-reducing sugars was performed by determining the difference between total sugars and reducing sugars, with results expressed in g/L sucrose equivalents.

2.8 Sensory Analysis and Yield

For the sensory analysis of the coffee samples, a panel of a certified Q-grader taster and two (2) SCA cuppers was employed. The samples were prepared and evaluated in accordance with the Specialty Coffee Association of America (SCAA) protocol at the LABCAFE coffee laboratory of the University of Tolima. This study conducted a comprehensive assessment of various attributes, including fragrance, aroma, flavor, residual aftertaste, acidity, body, uniformity, balance, clean cup, sweetness, defects, and the overall score (Specialty Coffee Association of America - SCAA, 2015). Yield was determined using a modified method based on the approach described by Donovan et al. (2019). In this method, 250.00 grams of parchment coffee

were weighed (400.00 grams for Natural coffee), followed by a series of processes involving threshing (C-200, Quantik, Colombia) and the manual selection of excelsa coffee, removing defective beans. After determining the final mass, it was divided by the initial mass to calculate the yield of the sample.

2.9 Statistical Analysis

The statistical analysis was performed using Infostat/L (Argentina, 1998), R-Studio (EEUU, 2011) and GraphPad Prism 5 software (EEUU, 2007). The following statistical methods were employed in this study: Analysis of Variance (ANOVA) with a significant threshold of $p < 0.05$, Kruskal-Wallis nonparametric analysis of variance ($p < 0.05$), and sample-based statistical inference tests; instead of Shapiro-Wilk normality test, Lilliefors normality test were used ($p < 0.05$) and the Student's t-test ($p < 0.05$). The Tukey test ($p < 0.05$) was utilized to identify statistically significant differences in the quantification of total sugars, reducing sugars (-Log10 to meet the normality assumption), non-reducing sugars and the sensory score.

3 RESULTS

3.1 Coffee Processing

Regarding the physicochemical characteristics of the coffee derived from leachate collected at the end of fermentation process, the data presented in Table 1 were obtained. This study considered the variation of each treatment over the studied time periods. The initial measurements for pH and acidity were recorded at 5.19 and 0.30%, respectively. The findings indicated that there were no statistically significant differences between the various treatments concerning pH and acidity levels. This conclusion was further supported by a Student's t-test, which yielded a p-value greater than 0.05.

Compared to the non-inoculated control, treatments inoculated with *P. kudriazevii* exhibited a more significant

decrease in pH during fermentation. This maintained an acidic environment favorable for yeast metabolism (around pH 3.80–4.00). The yeast consumed available sugars and produced metabolites and organic acids, including acetic acid. The concentration of acetic acid in the leachate ranged from 0.601% to 0.941%. These levels are consistent with previous reports on coffee fermentations and can modulate microbial metabolism (Avallone et al., 2001; Kwak et al., 2018; Pregolini et al., 2021). In this study, after 72.00 hours of fermentation, the TWI treatment reached a pH of 3.80 and a titratable acidity of 0.84%, whereas the NC treatment after 48.00 hours showed a higher pH (3.98) and lower acidity (0.601%).

3.2 Quantification of total sugars, reducing and non-reducing sugars

As illustrated in Table 2, a comprehensive summary of the data obtained from the quantification of total, reducing, and non-reducing sugars over time is presented. For total sugars, the calibration curve exhibited an optimal fit to the data, with a correlation coefficient of 0.9954, while for reducing sugars, the data exhibited a highly significant fit to the model, yielding an R^2 value of 0.9997.

When expressed as a percentage of the initial total sugars consumed, the inoculated treatments exhibited a range of consumption from 73.15% to 81.46% (initial 86.66 g/L to a range of 16.06-23.26 g/L), whereas the non-inoculated control treatments demonstrated a range of 71.35% to 73.62% (initial 86.66 g/L to a range of 22.86-24.68 g/L). These percentages were derived from the sugar concentrations reported in Table 2 and are consistent with those described by Puerta (2013), who documented an 80.70% depletion of initial total sugars after 74 hours of fermentation. Non-reducing sugars, calculated by difference and expressed as sucrose equivalents (g/L), did not demonstrate statistically significant changes across the evaluated factors. In certain treatment modalities, these values exhibited minimal fluctuations over time.

Table 1: Physicochemical Characteristics of Coffee Extracted from Leachate Produced During Fermentation.

Treatment	Fermentation time (h)	pH	Acidity (%)
Natural control (NC)	48.00	3.98	0.62 ± 0.03
	72.00	3.94	0.80 ± 0.03
Natural inoculated (NI)	48.00	3.92	0.62 ± 0.03
	72.00	3.85	0.76 ± 0.06
Traditional Washed Control (TWC)	48.00	3.96	0.68 ± 0.03
	72.00	3.87	0.94 ± 0.09
Traditional Washed Inoculated (TWI)	48.00	3.84	0.78 ± 0.33
	72.00	3.80	0.84 ± 0.12

Table 2: Quantification of sugars.

Treatment	Fermentation time (h)	Concentration of total sugars g/L	Concentration of reducing sugar g/L	Concentration of non-reducing sugars g/L
Coffee Cherry	0	86.66 ^a ± 6.97	72.56 ^a ± 1.45	14.10 ^{ab} ± 5.57
Natural Control	48.00	22.86 ^b ± 1.49	10.98 ^b ± 0.18	11.88 ^{ab} ± 1.63
	72.00	23.51 ^b ± 2.58	9.77 ^d ± 0.21	13.74 ^{ab} ± 2.71
Natural Inoculated	48.00	16.67 ^c ± 1.23	9.17 ^d ± 0.09	7.51 ^b ± 1.22
	72.00	16.06 ^c ± 1.10	8.53 ^e ± 0.20	7.53 ^b ± 1.25
Traditional Washed Control	48.00	24.83 ^b ± 1.10	10.93 ^b ± 0.16	13.90 ^{ab} ± 0.94
	72.00	24.68 ^b ± 3.23	8.98 ^d ± 0.14	15.70 ^a ± 3.20
Traditional Washed Inoculated	48.00	22.47 ^b ± 0.83	8.42 ^e ± 0.16	14.05 ^{ab} ± 0.74
	72.00	23.26 ^b ± 1.47	6.93 ^f ± 0.08	16.34 ^a ± 1.46

Note: Columns sharing a common letter are not significantly different ($p > 0.05$).

Notwithstanding, the lowest levels of non-reducing sugars (sucrose equivalents, g/L) were observed in the inoculated treatments (Figure 2), thereby supporting the role of yeast metabolism in the hydrolysis of macromolecules and in the formation of key aroma precursors such as reducing sugars, amino acids, and chlorogenic acids (Lee et al., 2015). As reported by Elhalis et al. (2021), the concentration of reducing sugars in the inoculated treatments was lower than that in the control treatments, indicating significant differences. The inoculated treatments demonstrated a reduction in reducing sugars ranging from 87.37% to 90.46% of the initial level, while the control treatments exhibited a reduction of 84.86% to 87.62%. Furthermore, the duration of coffee processing and fermentation influenced reducing sugar consumption, which increased with extended fermentation times, particularly in the traditional washed processing. In accordance with the findings of other studies, research indicates that reductions in sugars do not undergo complete degradation over time (Puerta, 2013; Vaz et al., 2023). This phenomenon occurs because polysaccharides are subject to degradation into simpler sugars, such as glucose and fructose, through a combination of enzymatic and hydrolytic reactions during the fermentation process (Ribeiro et al., 2017).

A significant positive correlation ($p < 0.05$) was observed between reducing sugars and total sugars during the coffee fermentation process ($R = 0.9896$).

3.3 Sensory Analysis and Yield

The results of the SCA scores for each treatment are presented in Table 3. Notably, significant differences were observed in the type of processing, with the Natural treatment achieving the highest SCA score. The Natural control treatment, which included 48 hours of fermentation, recorded an SCA score of 86.13. This treatment exhibited a fragrance and aroma profile characterized by notes of maple syrup and almond, while its flavor profile included hints of chocolate, almond, milk and vineyard.

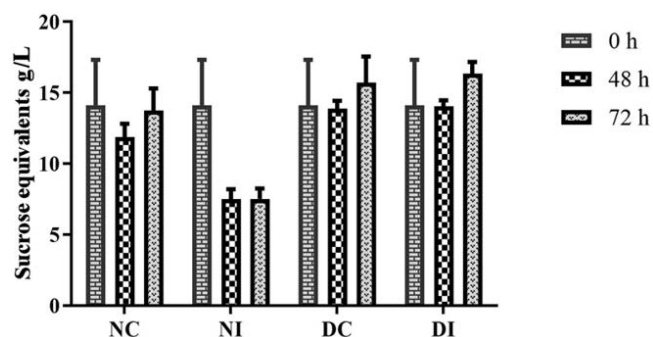


Figure 2: Temporal Concentration of Non-Reducing Sugars (Self-Authored).

Certain treatments inoculated with the strain exhibited fruity notes, including blueberry, orange, strawberry and pineapple, alongside alcoholic notes reminiscent of wine. These flavor profiles align with those reported by Shankar et al. (2022), who identified sweet notes associated with honey, berries and chocolate when utilizing *P. kudriavzevii*. The genus *Pichia* is typically linked to floral, vinegary, sweet and fruity characteristics (Pregolini et al., 2021). Notably, the 48.00 h inoculated washed treatment recorded an SCA score of 83.13, while all other inoculated treatments achieved SCA scores exceeding 84.00. This represents an improvement over the score of 81.50 reported by Elhalis et al. (2021) for coffee fermented with *P. kudriavzevii*.

The coffee yield varied between 50.95% and 77.72%. The lower yields were associated with Natural processing methods, primarily attributed to the presence of husk and pulp. It is important to highlight that the calculation of the yield factor at the national level influences the price that cooperatives offer to farmers for their coffee, with a reference score of 94.00 being the highest established (Pabón Usaquén & Osorio Pérez, 2018). To calculate this factor, a sample of 100.00 g of green coffee is typically utilized, from which defective beans are removed. Subsequently, the weight of the defective beans is subtracted from the initial mass.

Table 3: Sensory Evaluation Results and Yield.

Treatment	Fermentation time (h)	SCA score	Yield (%)	Cup profile
Natural	48.00	86.08 ^a ± 0.38	50.95	Winey, chocolate, almond, milk
Control	72.00	83.83 ^{bc} ± 0.38	50.95	Cocoa, caramelized, orange
Natural Inoculated	48.00	84.58 ^b ± 0.14	51.47	Black tea, brown sugar, blueberry
	72.00	84.25 ^b ± 0.25	51.20	Chocolate, strawberry, toasted almond
Washed	48.00	83.75 ^{bc} ± 0.51	77.72	Cocoa, vanilla, blueberry
Control	72.00	83.58 ^{bc} ± 0.14	75.08	Orange, lemon, caramelized
Washed	48.00	83.08 ^c ± 0.38	76.40	Cocoa, red fruits, caramelized
Inoculated	72.00	84.33 ^b ± 0.63	76.68	Chocolate, toasted almond, blueberry

Note: Columns sharing a common letter are not significantly different ($p > 0.05$).

A statistically significant positive correlation ($p < 0.05$) was observed between the content of reducing sugars and the SCA score during coffee fermentation ($R = 0.3100$). Elevated levels of reducing sugars may contribute to achieving a higher SCA score.

4 DISCUSSION

The initial pH value of 5.19 observed in this study aligns with prior reports indicating that coffee beans generally exhibit initial pH values ranging from 5.00 to 7.00 (Haile & Kang, 2019a; Kwak *et al.*, 2018). Fermentation time, temperature, and environmental conditions have been shown to markedly influence pH reduction (Vaz *et al.*, 2023). In the present study, post-fermentation pH values ranged from 3.80 to 3.98 across treatments. However, Jackels and Jackels (2005) emphasize the importance of maintaining pH values above 4.00 to produce a high-quality final beverage. The decrease in pH is commonly attributed to the formation and accumulation of organic acids, which can affect sensory quality. Acetic acid has been associated with defective beans and the development of undesirable odors and flavors (Peñuela-Martínez *et al.*, 2018). Therefore, controlling fermentation conditions is crucial given that acetic acid is frequently identified as one of the main acids in coffee fermentations, along with chlorogenic acids (Bressani *et al.*, 2018; Evangelista *et al.*, 2014). Also, studies have reported improvements in the sensory quality of coffee, as reflected in SCA scores, at pH values close to 4.00 (up to 85.00 SCA score), but not below this value as SCA scores were tending to decrease (Peñuela-Martínez *et al.*, 2018; Shankar *et al.*, 2022), which in this case resemble the pH values observed in treatments with 48.00 h of fermentation, compared to the results at 72.00 h.

In line with these physicochemical changes, adding an inoculum in the present study also influenced the coffee's sensory quality. Our results support the idea that fermentation is an independent processing step between fruit handling and drying. This step can be carried out with or without a defined starter culture to ensure consistent quality and reduce

economic losses (Carvalho *et al.*, 2023). Using a starter culture complements the natural microbial community and steers the fermentation process toward producing more uniform, predictable coffee beans with specific quality attributes (Haile & Kang, 2019a). However, Evangelista *et al.* (2014) have noted that the performance of starter cultures can vary depending on the coffee variety and processing method. This variability was evident in the present study, where the natural fermentation method produced sensory characteristics not observed in traditional washed treatments.

In this study, the reduction observed in reducing sugars was greater than that recorded for non-reducing sugars, suggesting that the microbial consortium preferentially metabolized simpler carbohydrates rather than hydrolysing more complex saccharidic structures (Avallone *et al.*, 2001). This phenomenon aligns with the findings of previous studies that have monitored the individual sugars present during coffee fermentation. These studies have reported that non-reducing sugars and other non-reducing carbohydrates undergo progressive hydrolysis, while the concentrations of glucose and fructose can exhibit transient increases as fermentation progresses (De Bruyn *et al.*, 2017; Haile & Kang, 2019b; Vaz *et al.*, 2023). While the quantification of specific sugars was not performed in the present study, the observed patterns for total, reducing, and non-reducing sugar fractions are consistent with the dynamic perspective of coffee fermentation, in which sugar consumption and generation are strongly dependent on time and microbial activity. Higher microbial loads have been demonstrated to expedite substrate consumption (Elhalis *et al.*, 2021), which is consistent with the accelerated depletion of sugar fractions observed in the inoculated treatments. In addition, Mahingsapun *et al.* (2022) found, among the trials conducted, that pH values decreased under all established conditions in 24 samples, from initial values of 5.71–7.39 to 4.28–6.05, with fermentation times ranging from 0.00 to 24.00 h, associated with greater sugar consumption, especially non-reducing sugars.

Research indicates that coffee varieties such as Bourbon, Catuaí, and Mundo Novo achieve higher SCA scores through spontaneous fermentation compared to those that utilize inoculated microorganisms (Bressani et al., 2021b; Carvalho Neto et al., 2017; Elhalis et al., 2021; Ribeiro et al., 2017), similar results were found with Castillo variety during this research, where the best treatment was Natural Control 48.00 h fermentation ($86.08^a \pm 0.38$ SCA points). This finding suggests that leveraging native microbiota can enhance coffee quality. Nevertheless, inoculation trials with *P. kudriavzevii* have yielded values ranging from 83.13 to 84.63 SCA points, which are considered ‘special–very good,’ results superior to those obtained using lactic acid bacteria and co-inoculations (Cassimiro et al., 2022). However, the variability in microorganism composition during coffee processing complicates the assurance of standardized quality, underscoring the necessity of exploring alternatives such as the implementation of starter cultures. Additionally, factors including geographical origin, climate, cultivar, post-harvest processing, and storage significantly impact product quality (Abreu et al., 2019; Figueiredo et al., 2019).

5 CONCLUSIONS

Sensory analysis indicated that *P. kudriavzevii* as a starter culture may modulate cup profile and contribute to process standardization, despite the absence of significant differences in the evaluated physicochemical variables. The Natural process also showed potential as an alternative for generating differentiated sensory profiles, based on SCA scores, compared with the Traditional Washed process. Consequently, controlled fermentations in GrainPro® bags and small-scale trials with selected yeasts are recommended to assess sensory effects and economic feasibility prior to large-scale application.

6 AUTHOR CONTRIBUTIONS

Conceptual idea: Leon, E.A.; Guarnizo, J.D.; Sandoval, A.P.; Cardona, Y.P.; Methodology design: Leon, E.A.; Guarnizo, J.D.; Sandoval, A.P.; Data collection: Leon, E.A.; Guarnizo, J.D.; Data analysis and interpretation: Leon, E.A.; Guarnizo, J.D.; Sandoval, A.P.; and Writing and editing: Leon, E.A.; Guarnizo, J.D.; Sandoval, A.P.; Cardona, Y.P.

7 DATA AVAILABILITY STATEMENT

Data available upon request to authors.

8 REFERENCES

- Abreu, G. F. et al. (2019). Raman spectroscopy: A new strategy for monitoring the quality of green coffee beans during storage. *Food Chemistry*, 287:241-248.
- Avallone, S. et al. (2001). Microbiological and biochemical study of coffee fermentation. *Current Microbiology*, 42(4):252-256.
- Ávila Núñez, R. et al. (2012). Contenido de azúcares totales, reductores y no reductores en Agave cocui Trelease. *Multiciencias*, 12(2):129-135.
- Bressani, A. P. P. et al. (2021a). Characterization of bioactive, chemical, and sensory compounds from fermented coffees with different yeasts species. *Food Research International*, 150:110755.
- Bressani, A. P. P. et al. (2021b). Influence of yeast inoculation on the quality of fermented coffee (*Coffea arabica* var. Mundo Novo) processed by Natural and pulped Natural processes. *International Journal of Food Microbiology*, 343:109107.
- Bressani, A. P. P. et al. (2018). Characteristics of fermented coffee inoculated with yeast starter cultures using different inoculation methods. *LWT*, 92:212-219.
- Carvalho F, L. J. et al. (2023). Coffee fermentation process: A review. *Food Research International*, 169:112793.
- Carvalho Neto, D. P. et al. (2017). Yeast Diversity and Physicochemical Characteristics Associated with Coffee Bean Fermentation from the Brazilian Cerrado Mineiro Region. *Fermentation*, 3(1):11.
- Cassimiro, D. M. de J. et al. (2022). Coinoculation of lactic acid bacteria and yeasts increases the quality of wet fermented Arabica coffee. *International Journal of Food Microbiology*, 369: 109627.
- Criollo Nuñez, J. et al. (2023). Effect of microencapsulated inoculum of *Pichia kudriavzevii* on the fermentation and sensory quality of cacao CCN51 genotype. *Journal of the Science of Food and Agriculture*, 103(5):2425-2435.
- Cruz-O’Byrne, R. et al. (2021). Microbial diversity associated with spontaneous coffee bean fermentation process and specialty coffee production in northern Colombia. *International Journal of Food Microbiology*, 354:109282.
- De Bruyn, F. et al. (2017). Exploring the impacts of postharvest processing on the microbiota and metabolite profiles during green coffee bean production. *Applied and Environmental Microbiology*, 83(1):e02398-16.
- Donovan, N. K. et al. (2019). Analysis of green coffee quality using hermetic bag storage. *Journal of Stored Products Research*, 80:1-9.

- Elhalis, H. et al. (2021). Microbiological and chemical characteristics of wet coffee fermentation inoculated with *Hansinaspota uvarum* and *Pichia kudriavzevii* and Their Impact on coffee sensory quality. *Frontiers in Microbiology*, 12:713969.
- Elhalis, H., Cox, J., & Zhao, J. (2023). Coffee fermentation: Expedition from traditional to controlled process and perspectives for industrialization. *Applied Food Research*, 3:100253.
- Evangelista, S. R. et al. (2014). Improvement of coffee beverage quality by using selected yeasts strains during the fermentation in dry process. *Food Research International*, 61:183-195.
- Figueiredo, L. P. et al. (2019). Raman spectroscopy for the differentiation of Arabic coffee genotypes. *Food Chemistry*, 288:262-267.
- Figueiredo, L. P. et al. (2015). Fatty acid profiles and parameters of quality of specialty coffees produced in different Brazilian regions. *African Journal of Agricultural Research*, 10(35):3484-3493.
- GraphPad Prism version 5.00 for Windows. San Diego, California, USA: GraphPad Software Inc.; 2007.
- Haile, M., & Kang, W. H. (2019a). Antioxidant activity, total polyphenol, flavonoid and tannin contents of fermented green coffee beans with selected yeasts. *Fermentation*, 5(1):29.
- Haile, M., & Kang, W. H. (2019b). the role of microbes in coffee fermentation and their impact on coffee quality. *Journal of Food Quality*, Article ID 4836709, 6 pages.
- Hameed, A. et al. (2018). Farm to consumer: factors affecting the organoleptic characteristics of coffee. II: postharvest processing factors. *Comprehensive Reviews in Food Science and Food Safety*, 17(5):1184-1237.
- InfoStat. InfoStat/L. Versión para Windows. Córdoba, Argentina: Facultad de Ciencias Agropecuarias, Universidad Nacional de Córdoba; 1998, <https://www.infostat.com.ar/>
- International Coffee Organization - ICO. (2021). *Trade statistics tables international coffee organization*. Available in: <https://ico.org/resources/trade-statistics-tables/>.
- Jackels, S. C., & Jackels, C. F. (2005). Characterization of the coffee mucilage fermentation process using chemical indicators: A field study in Nicaragua. *Journal of Food Science*, 70(5): C321-C325.
- Kwak, H. S. et al. (2018). Effect of yeast fermentation of green coffee beans on antioxidant activity and consumer acceptability. *Journal of Food Quality*, 5967130, 8 pages.
- Lee, L. W. et al. (2015). Coffee fermentation and flavor - An intricate and delicate relationship. *Food Chemistry*, 185:182-191.
- López-Legarda, X. et al. (2017). Comparación de métodos que utilizan ácido sulfúrico para la determinación de azúcares totales. *Revista Cubana de Química*, 29(2):2224-5421.
- Mahingsapun, R. et al. (2022). Enhancement of Arabica coffee quality with selected potential microbial iniciadores culture under controlled fermentation in wet process. *Food Bioscience*, 48:101819.
- Pabón Usaquén, J. P., & Osorio Pérez, V. (2018). Factores e indicadores de la calidad física, sensorial y sanitaria del café. Cenicafé. <https://biblioteca.cenicafe.org/bitstream/10778/4227/1/Cap07.pdf>
- Peñuela-Martínez, A. E. et al. (2018). Performance of different fermentation methods and the effect on coffee quality (*Coffea arabica* L.). *Coffee Science*, 13(4):465-476.
- Pregolini, V. B. et al. (2021). Influence of environmental microbiota on the activity and metabolism of starter cultures used in coffee beans fermentation. *Fermentation*, 7(4):278.
- Puerta, G. I. (2010). Fundamentos del proceso de fermentación en el beneficio del café. *Cenicafé*, 12. <https://biblioteca.cenicafe.org/bitstream/10778/345/1/avt0402.pdf>
- Puerta, G. I. (2013). Cinética química de la fermentación del mucilago de café a temperatura ambiente. *Cenicafé*, 64(1):42-59.
- Ribeiro, L. S. et al. (2017). Behavior of yeast inoculated during semi-dry coffee fermentation and the effect on chemical and sensorial properties of the final beverage. *Food Research International*, 92:26-32.
- RStudio Team. RStudio: Integrated Development Environment for R. Boston, MA: RStudio, PBC; 2011. Available in: <http://www.rstudio.com/>
- Santos Scholz, M. B. et al. (2018). The typicity of coffees from different terroirs determined by groups of physico-chemical and sensory variables and multiple factor analysis. *Food Research International*, 114:72-80.
- Silva Vale, A. et al. (2021). Facility-specific 'house' microbiome ensures the maintenance of functional microbial communities into coffee beans fermentation: implications for source tracking. *Environmental Microbiology Reports*, 13(4):470-481.

- Shankar, R. S. et al. (2022). Microbial ecology and functional coffee fermentation dynamics with *Pichia kudriavzevii*. *Food Microbiology*, 105:104012.
- Souza, M. L. et al. (2017). Use of wild yeasts as a biocontrol agent against toxigenic fungi and OTA production. *Acta Scientiarum. Agronomy*, 39(3):349-358.
- Specialty Coffee Association of America - SCAA. (2015). SCAA Protocols: Cupping Specialty Coffee. <https://icoff.ee/fa/wp-content/uploads/2017/05/cupping-protocols-SCAA.pdf>
- Vaz, C. J. T. et al. (2023). Effect of fermentation on the physicochemical characteristics and sensory quality of Arabica coffee. *3 Biotech*, 13:403.