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DOI: http://dx.doi.org/10.1590/1807-1929/agriambi.v28n1e272094 ORIGINAL ARTICLE

Inoculation of *Saccharomyces cerevisiae* with sugar cane juice as a starter culture in coffee (*Coffea arabica*) fermentation[1]

Inoculação de *Saccharomyces cerevisiae* com caldo de cana-de-açúcar como cultura iniciadora na fermentação do café (*Coffea arabica*)

Wilmer L. Ladino-Garzon[2], Yeison F. Barrios-Rodríguez[2,3] & Claudia M. Amorocho-Cruz[2*]

[1] Research developed at Universidad Surcolombiana/South Colombian Coffee Research Center/Department of Agricultural Engineering, Neiva, Huila, Colombia
[2] Universidad Surcolombiana/South Colombian Coffee Research Center/Department of Agricultural Engineering, Neiva, Huila, Colombia
[3] Universitat Politécnica de València/Instituto Universitario de Ingeniería de Alimentos, Valencia, Spain

HIGHLIGHTS:

Yeast from wine production is an alternative to inoculation as a starter in coffee fermentation chaptalized with sugar cane.
Treatments with sugar cane and S. cerevisiae *are a proposal that contributes to the production of specialty coffee.*
The incorporation of additives such as sugar can favor the metabolism of yeast because it is a source of carbohydrates.

ABSTRACT: This study aims to evaluate the effect of sugarcane juice and the addition of commercial yeast *Saccharomyces cerevisiae* var. *bayanus* (≥ 1 × 1010 cfu/g) during the fermentation of coffee to the beverage's sensory characteristics and the coffee bean's chemical composition. A completely randomized experimental design with two replicates is carried out for four treatments, distributed as follows: i) water addition (0.78 kg), ii) sugar cane juice addition (0.78 kg), iii) sugar cane juice addition (0.78 kg) combined with yeast Oenoferm® Freddo (0.12 g) and iv) sugar cane juice addition (0.78 kg) combined with yeast Oenoferm® Color (0.12 g). After fermentation and drying, the samples were subjected to medium roasting and analyzed using infrared spectroscopy and sensory analysis according to the methodology of the Specialty Coffee Association. The implementation of organic additives directly affected the attributes and sensory notes, allowing coffee to be classified as a specialty coffee with a score above 80 points. Adding sugar cane juice or a combination of sugar cane juice and *Saccharomyces cerevisiae* showed promising results in improving coffee beverage quality. Additionally, chemometric analysis of the infrared spectrum showed that the chemical characteristics of roasted coffee were affected, which correlated with the sensory results. The addition of cane juice only (T2) and the Oenoferm® Freddo yeast strain (T3) presented the best sensory quality.

Key words: Guarapo, coffee postharvest, yeast inoculation, infrared spectroscopy

RESUMO: O objetivo deste estudo foi avaliar o efeito do caldo de cana-de-açúcar e da adição levedura comercial *Saccharomyces cerevisiae* var. *bayanus* (≥ 1 × 1010 cfu/g) durante a fermentação do café nas características sensoriais da bebida e na composição química do grão de café. Foi realizado um experimento completamente aleatório com duas repetições para quatro tratamentos, distribuídos da seguinte forma: i) adição de água (0.78 kg), ii) adição de caldo de cana-de-açúcar (0.78 kg), iii) adição de caldo de cana-de-açúcar (0.78 kg) combinado com a levedura Oenoferm® Freddo (0.78 kg) e iv) adição de caldo de cana-de-açúcar (0.78 kg) combinado com a levedura Oenoferm® Color (0.78 kg). Após fermentação e a secagem, as amostras sáo submetidas à torrefação média e analisadas por espectroscopia de infravermelho e análise sensorial de acordo com a metodologia da Specialty Coffee Association. A implementação de aditivos orgânicos afetou diretamente os atributos e as notas sensoriais, permitindo que o café fosse classificado como um café especial com uma pontuação acima de 80 pontos. A adição de suco de cana-de-açúcar ou de uma combinação de suco de cana-de-açúcar e *Saccharomyces cerevisiae* apresentou resultados promissores na melhorar da qualidade da bebida do cafe. Além disso, a análise quimiométrica do espectro infravermelho mostrou que as características químicas do café torrado foram afetadas, o que se correlacionou com os resultados sensoriais. A adição apenas de suco de cana (T2) e da cepa de levedura Oenoferm® Freddo (T3) apresentou a melhor qualidade sensorial.

Palavras-chave: Guarapo, pós-colheita de café, inoculação de leveduras, espectroscopia de infravermelho

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* Corresponding author - E-mail: claudiamilena.amorocho@usco.edu.co
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Introduction

The quality of coffee is a differentiating factor during commercialization and can be determined mainly by the fermentation stage (Pereira et al., 2020). During this process, microorganisms (yeasts and bacteria) contribute to mucilage degradation, suppress fungal growth, and influence the sensory quality of the beverage (Haile & Hee, 2019). This has allowed the introduction of modifications to the fermentation process by inoculating microorganisms that provide positive sensory characteristics to optimize the coffee production process (Pereira et al., 2020).

The implementation of yeast starter cultures in coffee fermentation allows for the modulation of aromatic and flavor attributes in the beverage (Martínez et al., 2017; Pereira et al., 2018; Da Mota et al., 2020; Elhalis et al., 2023), allowing the standardization and control of fermentation processes (Lee et al., 2015). Among the starter cultures, *Saccharomyces cerevisiae* inoculation positively affects the beverages' sensory characteristics (Pereira et al., 2018; Da Mota et al., 2020; Bressani et al., 2021). Moreover, it has been observed to favor antioxidant activity, total flavonoid content, and total polyphenol content (Kwak et al., 2018).

Sugarcane juice is an exciting alternative as an additive for yeast growth because of its high carbohydrate content, which is an essential source for the metabolism of these microorganisms (Panigrahi et al., 2021). Among the carbohydrates present in sugarcane juice, the most common monosaccharides are glucose and fructose, whereas the most common disaccharide is sucrose (Arif et al., 2019). Considering that the sensory quality attributes of coffee can be enhanced from the perspective of induced fermentation in the wet postharvest period, this study aimed to evaluate the incidence of *Saccharomyces cerevisiae* starter cultures and the addition of sugar cane juice during coffee fermentation on the sensory quality of the beverage and on the chemical composition of roasted coffee beans.

Materials and Methods

Forty kilograms of cherry coffee (*Coffea arabica L.*) variety Colombia, obtained in Huila (2°20'31"N, 75°29'38"W and altitude of 1650 masl), Colombia, were collected. This sample was preserved for 4 hours in expanded polystyrene containers and cooling gels (4.0 °C) until processing. Before pulping, the cherry coffee was washed with potable water, and impurities were discarded by density. The pulp was then removed using a pulper with a cylindrical grading sieve without adding water.

Oenoferm® Freddo (Erbslöh España, Spain) and Oenoferm® Color (Erbslöh España, Spain) reference strains of *Saccharomyces cerevisiae* var. *bayanus* were reactivated in sugar cane juice at a 50% dose, according to the manufacturer's specifications. The incubation conditions were 30 °C and 80% relative humidity for 24 hours in a climatic chamber (Memmert HPP 110, Germany), emulating farm environmental conditions. Previously, the sugar cane juice was subjected to heat treatment of 121 °C for 15 min (Sci Finetech FTAC-705P, Korea). A completely randomized experimental design with two replicates was carried out and four treatments were established: control treatment with the addition of water (T1), treatment with the addition of sugar cane juice without yeast (T2), treatment with the addition of sugar cane juice and Oenoferm® Freddo yeast strains (T3), and finally treatment with the addition of sugar cane juice and Oenoferm® Color yeast (T4). In each experimental unit, 2.6 kg of pulped coffee were used. The addition of water or sugar cane juice (0.78 kg) used in the fermentation process was carried out with a proportion of 30% (wet mass proportion) of the coffee mass (2.6 kg) to be fermented (Puerta-Quintero, 2012). Fermentation was carried out for 24 hours in hermetically sealed sterile polyethylene bags in a climatic chamber (Memmert HPP 110, Germany) at 22 °C and 70% relative humidity. Finally, the coffee mass was washed three times with distilled water and subjected to solar drying until it reached a humidity range of 9–10% (Velásquez et al., 2018).

At the beginning and end of the fermentation process, 100 g of samples (liquid mass of fermentation) were obtained to determine the physicochemical parameters of soluble solids, titratable acidity, and pH. Soluble solids were determined by the indirect method of a refractometer taking 0.5 mL of mucilage (Atago PR-201α, USA) (Peñuela-Martínez, 2021). The percentage of the total titratable acidity was expressed as lactic acid, for which 20 mL of mucilage was taken and 0.06 mL of phenolphthalein was added, followed by titration with an alkaline solution of sodium hydroxide (0.1 N) until a pH of 8.1 was reached (Gallego & Rodríguez, 2021). Finally, the pH was determined using a potentiometer with 20 mL of mucilage at a temperature of 25 °C (Peñuela-Martínez, 2021).

It were sampled 10 g during the liquid mass of the fermentation process at the beginning and end (0 and 24 hours) and homogenized in 90 mL of buffered peptone water (BPW, 3M Health Care, Germany). Subsequently, serial dilutions were made for seeding on the potato dextrose agar plate (PDA, HiMedia Laboratories Pvt. Ltd., India), incubating at 30 °C for 48 hours. After incubation, time had elapsed, and viable colony-forming units (CFU) were counted (Martínez et al., 2017).

Spectral measurements were performed following the methodology of Barrios-Rodríguez et al. (2020) with some modifications. A Cary 630 FTIR spectrometer (Agilent Technologies, Santa Clara, CA, USA) with a DLATGS detector and ATR sampler accessory was employed between wavelengths of 4000–650 cm^{-1}, with a resolution of 8 cm^{-1} and 32 scans. The final spectrum was obtained from the average of three measurements. Approximately 1.0 mg, readings were taken for the caffeine and chlorogenic acid standards. Approximately 1.0 g of roasted ground coffee passed through sieve No. 30 and was retained on No. 40 ($600 > D > 426$ μm) under ambient conditions of 58% relative humidity and 23 °C. All determinations were performed on roasted ground coffee and the standards were in the powder with a purity of ≥99% (chlorogenic acids) and ≥95% (caffeine).

Sensory analysis was performed by a panel Q-grader of five coffee tasters, according to the cupping protocol described by the Specialty Coffee Association-SCA. Ten quality variables were evaluated: fragrance/aroma, flavor, aftertaste, acidity, body, balance, uniformity, clean cup, sweetness, and overall score using a scale of 6.00 to 9.75. Each taster evaluated five cups per treatment and the final score was calculated as the sum

of the attributes considered. Descriptive notes were recorded and grouped hierarchically according to the variables stipulated in the flavor wheel (Spencer et al., 2016).

Simple ANOVA evaluated the physicochemical results of each fermentation time variables, and Fisher's LSD test was performed at $p \leq 0.05$ using Statgraphics - Centurion XVI software. The infrared spectral data were pretreated with baseline and second-derivative corrections. This processing was performed using the R program with the ChemoSpec, mdatools, and ChemoSpecutils libraries. Subsequently, a principal component analysis (PCA) was performed on the region between 650 and 1800 cm^{-1}. For the PCA, the data were initially scaled and centered to obtain a mean of zero and a standard deviation of one. In addition, control statistics such as residual sum squares (RSS) and Hotelling's T^2 were used to detect and remove outliers from the experimental data. Finally, the information obtained from the first seven principal components was used to create a hierarchical cluster. The components were selected from the cumulative explained variance graph, choosing the number of components that make the curve begin to flatten significantly and explained more than 70% of the variability.

Results and Discussion

Sugarcane juice treatments showed a decrease in soluble solids at the end of the fermentation stage (Table 1). This may be because the carbohydrate content of sugarcane juice favors yeast metabolic activity. However, the inverse behavior was observed in Table 1, which can be attributed to the dissolution of the substances present in the mucilage with water. The dissolution of carbohydrates from the mucilage has represented increases between 5.9 and 8.0% of soluble solids in systems under the same experimental conditions (fermentation with water addition) (Puerta-Quintero 2012).

The initial pH values decreased after 24 hours of fermentation for all treatments (Table 1), which may be associated with the production of metabolites from the lactic acid bacteria naturally present in coffee beans. (Kwak et al., 2018). According to Triviño-Pineda et al. (2022), a decrease in pH may be due to i) the transformation of sugars to bioethanol, ii) CO_2 desorption, and iii) acid production. The total acidity (percentage of lactic acid) increases with the fermentation time, which is inversely related to the pH behavior, and may be directly related to microbial growth in coffee mash (Kwak et al., 2018). The treatments inoculated with *Saccharomyces cerevisiae* did not present statistically significant differences ($p < 0.05$) (Table 1); however, significant differences ($p>0.05$) were observed between the treatments not inoculated with microorganisms (T1 and T2). The highest percentage of lactic acid was observed in the treatments with the addition of sugarcane juice, which suggests that the monosaccharides, disaccharides, and trisaccharides of this substrate contribute to the production of metabolites and acidification.

In the viable cell count, a higher population was observed in the treatments with the addition of yeast and sugarcane juice (Table 1), which allowed us to consider that this combination was effective for a more significant proliferation of these microorganisms in the fermentation stage. At the beginning of fermentation, the control treatment with the addition of sugarcane juice did not show statistically significant differences ($p>0.05$) from the control treatment (T1) or the yeast inoculation treatments (T3 and T4) (Table 1). At the end of the fermentation stage, the viable cell count showed a statistically significant difference ($p < 0.05$) between the treatments containing sugarcane juice (T2, T3, and T4) and the control treatment with water (T1). This could be related to the presence of carbohydrates, such as fructose, glucose, sucrose, and raffinose (Panigrahi et al., 2021), which provide suitable conditions for the development of the microbiota. The tendency to increase the viable cell count of treatments inoculated with Oenoferm® Freddo and Oenoferm® Color yeasts is consistent with that published by Kwak et al. (2018), who used commercial *Saccharomyces cerevisiae* strains for wine.

Finally, the results indicated that adding sugar cane juice affected the final physicochemical and microbiological parameters compared to the control treatment (T1). Sugar cane juice provides conditions that facilitate the growth of endogenous microorganisms or their inoculation into the coffee mass during fermentation.

Sensory analysis showed a positive effect of adding sugarcane juice combined with yeast on the final score awarded by the tasting panel (Table 2). Other studies have demonstrated the efficiency of using organic additives, such as yeasts (Pereira et al., 2018; Kwak et al., 2018; Da Mota et al., 2020), panela honey, lemongrass infusion, and efficient microorganisms (Ararat & Trujillo, 2018); to improve the sensory characteristics of the beverage. The results showed that using yeast and sugarcane juice in the fermentation process favored the cup quality of coffee, which can be used as an alternative to improve the quality and compete in specialized markets. The coffee with

Table 1. Physicochemical variables and cell viable counts

Treatments	Fermentation times (h)	Soluble solids (°Brix)	pH	Total acidity (% Lactic acid)	Cell viable counts (log CFU/mL)
T1	0	4.85±0.60a	4.86±0.03a	0.07±0.01a	7.74±0.64a
	24	6.43±0.05a	3.57±0.01a	0.97±0.05a	11.14±0.92a
T2	0	14.08±0.08b	5.00±0.01c	0.21±0.02b	8.81±1.00ab
	24	13.77±0.05b	3.65±0.01b	1.24±0.05c	12.10±0.00b
T3	0	14.52±0.12c	5.01±0.03c	0.21±0.02b	9.03±0.92b
	24	13.75±0.20b	3.65±0.02b	1.06±0.07b	>12.10±0.00c
T4	0	14.45±0.05bc	4.95±0.05b	0.20±0.02b	9.06±1.27b
	24	13.72±0.04b	3.65±0.01b	1.00±0.04ab	>12.10±0.00c

T1-control treatment with the addition of water; T2- treatment with the addition of sugar cane juice without yeast; T3- treatment with the addition of sugar cane juice and Oenoferm® Freddo yeast strains; T4- treatment with the addition of sugar cane juice and Oenoferm® Color yeast. Mean ± standard deviation (SD) Mean values in the same column with different letters indicate significant differences (LSD; $P < 0.05$)

water addition obtained the lowest score (73.88) compared to the other treatments with added sugarcane juice (82.87) or the combination of sugarcane juice and yeast (83.31 and 81.30), excelling with scores within the "Very good" specialty range according to the Specialty Coffee Association (SCA).

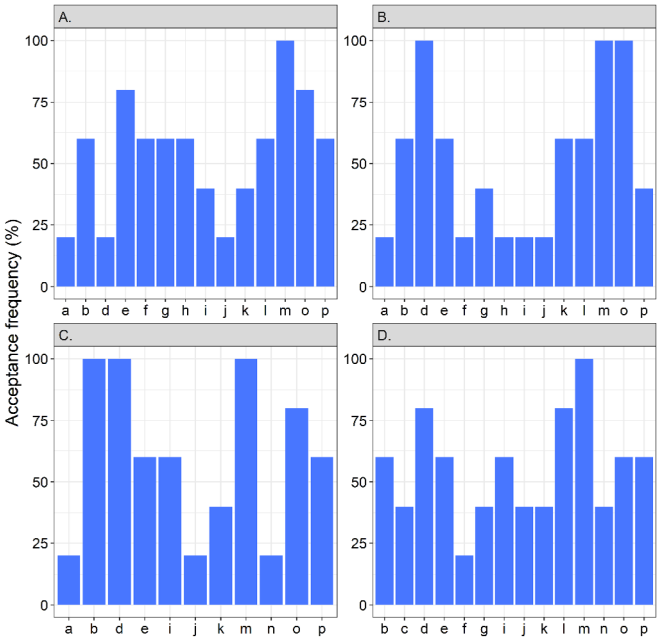
This indicates that sugarcane juice can be classified as a potential additive for improving the sensory quality of coffee. This is important because it is a resource that is available to coffee producers in rural areas. However, the sensory results obtained in the treatments with the addition of Oenoferm® Freddo and Oenoferm® Color yeasts demonstrate that by adding adequately selected starter microorganisms, coffee beverages that have great potential for attracting consumers can be obtained. Da Mota et al. (2020) found that the best cup result for pulped coffee was related to *Saccharomyces cerevisiae*, increasing up to five scores for the control.

The sensory descriptors provided by the panelists showed that treatments T3 and T4 presented a more significant presence of positive sensory attributes (Figure 1C and D). However, T2 presented very similar characteristics in terms of attributes but also showed undesirable notes such as plastic and chemicals (Table 3), whereas in the T1 treatment, unpleasant notes such as phenol, gasoline, and plastic stood out.

Positive sensory descriptors were shared by all treatments, such as fruity, lemon, orange, unfair, peanut, nutty, panel, caramel, herbal, lemon leaves, and astringent. This result indicates that these attributes are directly related to the raw material used and not to the treatment implemented. Additionally, the negative descriptors related to T2 were present at a lower percentage and intensity than those related to T1 (Figure 1B and A). This was reflected in the final cup score of T2, cataloged within the range of "Very good" specialty coffee.

Treatment T3 had the highest score (83.31 scores). This treatment was the only one that did not present negative scores related to alcohol/fermented or paper/mold. Therefore, it is possible that the addition of the Oenoferm® Freddo yeast strain positively influences the modulation of sensory descriptors in the coffee beverage.

In the treatments with the addition of Oenoferm® Freddo and Oenoferm® Color yeast strains, there was a more excellent perception of positive notes related to fruit, spices, sweetness, and aromatic herbs and no negative notes related to chemicals. The Oenoferm® Color (T4) strain provides ripe fruit, wood, spice, nut, and toasted profiles (Erbslöh, 2022b). These



Codes in capital letters refer to the hierarchy used in the flavor wheel (Spencer et al. 2016). a- Floral (Floral), b- Fruity (Berry), c - Fruity (Dried fruit), d - Fruity (Other fruit), d- Fruity (Citrus fruit), f- Sour/fermented (Alcohol/fermented), g- Other (Papery/musty), h- Other (Chemical), i- Spices (Pepper), j- Spices (Brown Spice), k- Nutty/Cocoa (Nutty), l- Nutty/Cocoa (Cocoa), m- Sweet (Brown Sugar), n- Sweet (Overall sweet), o- Aromatic herbals and Herbal, p- Under-ripe

Figure 1. Flavor wheel sensory attributes provided by judges and percentage of occurrence in samples analyzed to T1- control treatment with the addition of water (A); T2- treatment with the addition of sugar cane juice without yeast (B); T3- treatment with the addition of sugar cane juice and Oenoferm® Freddo yeast strains (C); T4- treatment with the addition of sugar cane juice and Oenoferm® Color yeast (D)

characteristics can be related to the attributes described by the tasting panel as strawberry, blackberry, fruity, cherry, green apple, citrus, lemon, orange, spicy, pepper, nutmeg, peanut, walnut, hazelnut, almond, dry, wood, and plum, highlighting that the dry and woody notes could be related to the coffee defects of the "earthy" group.

The perception of prunes is related to the "over-fermented" group of defects with the relationship to overripe flesh (Osorio, 2021). For the Oenoferm® Freddo yeast strain added to T3, the profile is related to fruity characteristics, citrus fruits, and green apple (Erbslöh, 2022a), which can be related to fruity notes, strawberry, blackberry, blackberry, cherry, lemon, orange, kiwi, and green apple perceived for samples of this treatment.

Table 2. Mean values of the attributes and final score for coffee according to Specialty Coffee Association

Flavor attribute	T1	T2	T3	T4
Fragrance/aroma	8.18±0.47a	8.25± 0.53a	8.23±0.58a	8.18±0.58a
Flavor	7.63±0.57a	8.04± 0.53a	8.05± 0.69a	7.80± 0.42a
Aftertaste	7.63±0.44a	7.85±0.49a	7.93±0.50a	7.70±0.45a
Acidity	7.00±0.41a	7.30±0.23b	7.33±0.26b	7.23±0.18ab
Body	7.08±0.39a	7.33±0.31a	7.28±0. 34a	7.23±0.25a
Uniformity	6.85±1.33a	9.80±0.42b	9.80±0.42b	9.50±0.85b
Balance	7.23±0.79a	7.58±0.83a	7.74±0.72a	7.45±0.75a
Clean cup	6.83±1.48a	9.80±0.42b	9.80±0.42b	9.50±0.85b
Sweetness	9.20±1.69a	10.00±0.00a	10.00±0.00a	9.90±0.32a
Overall	7.08±1.09a	7.13±0.34a	7.18±0.31a	7.03±0.22a
Final score	73.88±5.27a	82.87±2.03b	83.31±2.29b	81.30±2.34b

T1- control treatment with the addition of water; T2- treatment with the addition of sugar cane juice without yeast; T3- treatment with the addition of sugar cane juice and Oenoferm® Freddo yeast strains; T4- treatment with the addition of sugar cane juice and Oenoferm® Color yeast. Mean ± standard deviation (SD) Mean values in the same row with different letters indicate significant differences (LSD, $p \leq 0.05$)

Table 3. Grouping of notes found by treatment according to sensory attributes of the flavor wheel according to the descriptors proposed by Spencer et al. (2016)

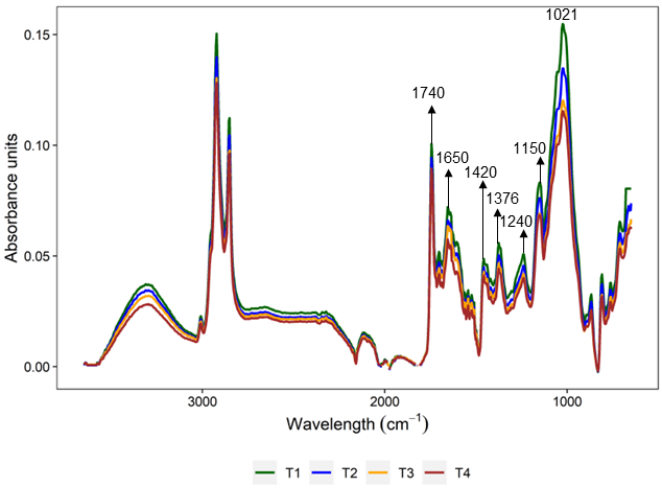
Category	Treatment			
	T1	T2	T3	T4
Floral /(Floral)	Floral	Floral	Floral	-
Fruity (Berry)	Red fruit	Strawberry, blackberry	Strawberry, blackberry	Strawberry, blackberry
Fruity (Dried fruit)	-	-	-	Prune
Fruity (Other fruit)	Fruity, apple	Fruity, kiwi, cherry.	Fruity, cherry, kiwi, green apple	Fruity, cherry, green apple
Fruity (Citrus fruit)	Lemon, orange	Lemon, orange	Lemon, orange	Citric, lemon, orange
Sour/fermented (Alcohol/fermented)	Pulpy (fermented)	Pulpy (fermented)	-	Pulpy (fermented)
Other (Paper/musty)	Phenolic	Woody	Woody	Woody
Other (Chemical)	Gasoline (Petroleum), plastic	Plastic, chemical	-	-
Spices (Pepper)	Spice, pepper	Spice	Spice, spicy, pepper	Spice, spicy, pepper
Spices (Brown spice)	Cinnamon	Cinnamon	Anise	Nutmeg
Nutty/cocoa (Nutty)	Peanut, nutty	Peanut, nutty	Peanut, nutty	Peanut, nutty, hazelnut, almond
Nutty/cocoa (Cocoa)	Chocolate	Chocolate	-	Chocolate
Sweet (Brown sugar)	Sweet, panela, caramelized	Panela, caramelized	Panela, caramelized, brown sugar	Panela, caramelized, brown sugar
Sweet (Overall sweet)	-	-	Sugar cane	Sugar cane
Herbals y Aromatics herbals.	Herbal, lemongrass, ginger, lemon leaves	Herbal, lemon leaves	Herbal, lemongrass, basil, lemon leaves	Herbal, fresh herbal, lemon leaves, lemongrass
Under-ripe	Astringent, under-ripe	Astringent	Astringent	Astringent, under-ripe

T1- control treatment with the addition of water; T2 - treatment with the addition of sugar cane juice without yeast; T3 - treatment with the addition of sugar cane juice and Oenoferm® Freddo yeast strains; T4- treatment with the addition of sugar cane juice and Oenoferm® Color yeast. A - Floral (Floral), b - Fruity (Berry), c - Fruity (Dried fruit), d - Fruity (Other fruit), d - Fruity (Citrus fruit), f - Sour/fermented (Alcohol/fermented), g - Other (Papery/musty), h - Other (Chemical), I - Spices (Pepper), j - Spices (Brown Spice), k - Nutty/Cocoa (Nutty), l - Nutty/Cocoa (Cocoa), m- Sweet (Brown Sugar), n - Sweet (Overall sweet), o - Aromatic herbals and Herbal, p - Under-ripe

Finally, the perception of sweet caramel was found in all treatments used, which could be related to the presence of benzacetaldehyde, which is also responsible for the fruity and floral notes in some treatments (Pereira et al., 2018). These last notes are also related to yeast fermentation by transforming 2-methyl-butanal and 3-methyl-butanal into alcohols, among which *Saccharomyces cerevisiae* var. *bayanus* (Duboc et al., 2003).

The effect of the treatments on the roasted coffee chemical composition was evaluated through the analysis of the mid-infrared spectrum (4000-650 cm^{-1}). According to the Lambert-Beer law, an increase in absorbance can represent a difference in the concentrations of the samples evaluated (Huang et al., 2021). The data showed evidence of changes in the absorbance values, mainly in the zone defined as the fingerprint area between 1800 and 650 cm-1 (Figure 2). Treatments without yeast (T1 and T2) presented the highest absorbance values, indicating that the addition of yeast during fermentation influenced the chemical composition (degrading or transforming compounds).

The wavenumbers of the higher incidence of the applied treatments were 1650 cm^{-1}, the region between 1420–1240 cm^{-1}, 1150 cm^{-1}, and 1021 cm^{-1}. The absorbance in the region 1660–1640 cm^{-1} region is related to the absorption of the cyclic amide caffeine and the vibration of its C=O bond (Craig et al., 2018). The band at 1150 cm^{-1} was related to the vibration of the C - O bond of the ether group of cellulose (Craig et al., 2018; Barrios-Rodríguez et al., 2020), whereas the wavelengths at 1420–1240 cm^{-1} were associated with the deformation of the O-H group of chlorogenic acids (Barrios-Rodríguez et al., 2020). In contrast, the absorbance at 1021 cm^{-1} was associated with the ester functional group C-O-C, which may be due to the presence of quinic acid in coffee, which belongs



T1- coffee + water, T2- coffee + sugar cane juice, T3- coffee + sugar cane juice + Oenoferm® Freddo yeast strain and T4- coffee + sugar cane juice + Oenoferm® Color yeast strain

Figure 2. Infrared spectrum of roasted coffee obtained by the different treatments used in fermentation

to the chlorogenic acid family (Jiménez-Ochoa et al., 2022). Barrios-Rodríguez et al. (2021) also related the band region corresponding to 1450–1250 cm^{-1} to chlorogenic acids.

To corroborate the presence of chlorogenic acids and caffeine in the different treatments, spectra of these compounds were obtained with purities of ≥99 and ≥95%, respectively. Figure 3A and B show the standard spectra of caffeine and chlorogenic acid, broadening the region from 1800 cm^{-1} to 650 cm^{-1}. Garrigues et al. (2000) and Gallignani et al. (2008) reported that for the characterization of caffeine, bands with intensities of 1710 cm^{-1}, 1659 cm^{-1} (originating from the vibration of carbonyl groups), and 1554 cm^{-1} are of the most significant interest. The authors highlight the importance of the region between 1650–1659 cm^{-1} because it does not show

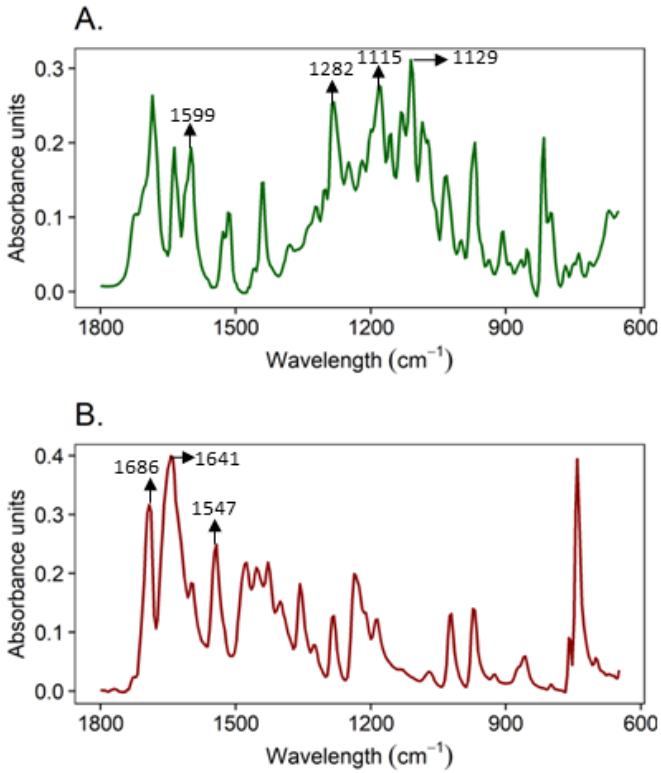
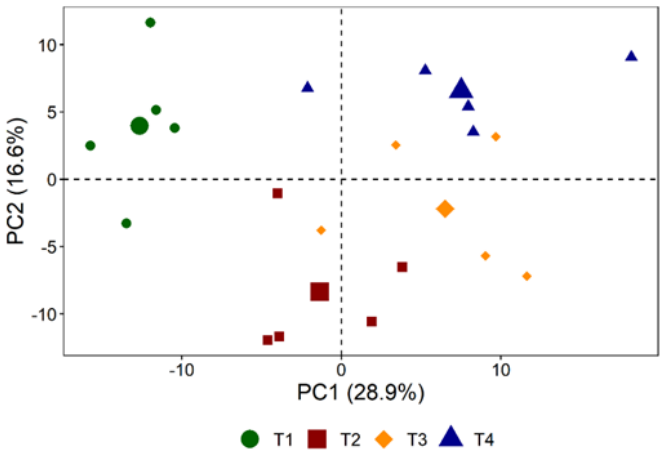


Figure 3. Extended ATR-FTIR spectrum in the region from 1800 cm^{-1} to 650 cm^{-1} for standard chlorogenic acids (bands of interest at 1282 cm^{-1}, 1115 cm^{-1} and 1129 cm^{-1}) (A), Caffeine (bands of interest at 1696 cm^{-1}, 1641 cm^{-1} and 1547 cm^{-1}) (B)

interference by other compounds. Ayala (2010) assigned the bands at 1700 cm^{-1} to C=N stretching, 1658 cm^{-1} to asymmetric C=O stretching, and 1548 cm^{-1} to the symmetric C=O stretching of caffeine. In the case of chlorogenic acid, Lee et al. (2015) described the following bands of interest for chlorogenic acid isomers 1605 cm^{-1}, 1276 cm^{-1}, 1165 cm^{-1}, and 1120 cm^{-1}. Therefore, the peaks at 1599 cm^{-1},1282 cm^{-1}, 1155 cm^{-1}, and 1129 cm^{-1} (Figure 3A) may be more relevant for the characterization of this compound. This information allowed for the association of the presence of caffeine and CGA in the analyzed samples and corroborated that the peaks observed (Figure 3A and B) corresponded to caffeine and chlorogenic acids in the different roasted coffee samples.

The results of the exploratory analysis performed by PCA where the second derivative of the data of the infrared spectrum showed a possible grouping of the treatments applied with, explanation of 45.5% of the total variability in the data with the first two components (Figure 4). The samples corresponding to T1 showed a clustering trend over the negative PC1 zone, whereas T2 was distributed over the negative PC2 zone. Most T4 samples were grouped in quadrant I of the graph, whereas T3 samples showed more significant variability and were grouped together with T2 and T4.

The information obtained from the infrared spectra could contribute to the grouping of treatments and help explain the effect of yeast and sugarcane juice addition on coffee fermentation. To exploit the information provided by the PCA and verify the similarity between the samples, a hierarchical cluster analysis was applied with the first seven principal components obtained from the PCA (>70%). The resulting



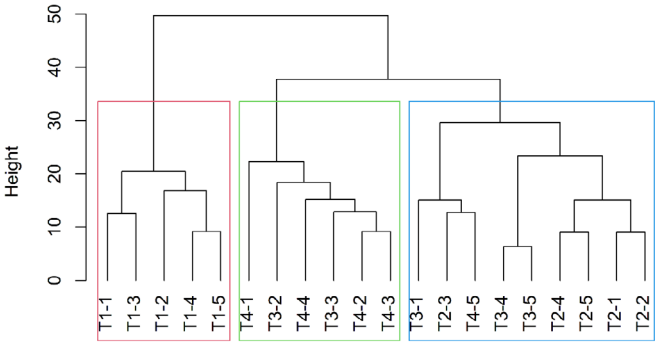
T1- control treatment with the addition of water; T2- treatment with the addition of sugar cane juice without yeast; T3- treatment with the addition of sugar cane juice and Oenoferm® Freddo yeast strains; T4- treatment with the addition of sugar cane juice and Oenoferm® Color yeast

Figure 4. Classification of treatments evaluated by PCA for data obtained by second derivative in the infrared spectral range 650–1800 cm^{-1}

dendrogram is shown in Figure 5, where three groups can be observed: i) samples T1, ii) samples T4, and iii) samples T3 and T2, thus confirming what was indicated by the PCA results.

T1 (control treatment with the addition of water) presented chemical characteristics different from those of the other treatments with the addition of sugar cane juice and yeast, indicating an effect of sugar cane juice on the final chemical composition of the coffee beans. Sugar cane juice may favor the growth of acidophilic microorganisms, such as lactic acid bacteria and yeasts, which could affect the characteristics of coffee beans (Panigrahi et al., 2021). These microorganisms present in the slime coffee mass, generate other compounds through their metabolic pathways, such as lactic acid, organic acids, gums, ethanol, oligosaccharides, and polysaccharides, as well as the hydrolysis of sucrose to generate fructose and glucose (Elhalis et al., 2020; Panigrahi et al., 2021).

The samples treated with the Oenoferm® Color yeast strain (T4) showed a different clustering compared to the other treatments, whereas treatment with the addition of cane juice only (T2) and that with the Oenoferm® Freddo yeast strain (T3)



T1- control treatment with the addition of water; T2- treatment with the addition of sugar cane juice without yeast; T3- treatment with the addition of sugar cane juice and Oenoferm® Freddo yeast strains; T4- treatment with the addition of sugar cane juice and Oenoferm® Color yeast

Figure 5. Cluster analysis for the classification of samples according to the treatment applied to coffee beans during fermentation

were grouped in the same cluster (Figure 5). The behavior of T3 could be related to the development temperature conditions of the experimental phase (22 °C) and the intrinsic characteristics of the strain. This strain tolerates cold conditions, with optimal development between 13 and 17 °C (Erbslöh, 2022a). Because the experiment did not present optimal conditions for the growth of this yeast, the microbial development phases (adaptation, exponential, stationary, and death) could have been affected, diminishing its effect during fermentation in coffee beans, generating variability in information, and maintaining similar characteristics to the treatment with sugar cane juice (T2). In contrast, the Oenoferm® color yeast used in T4 presents optimal growth conditions between 18 and 28 °C, which could be favored during fermentation, exerting an effect on the chemical composition of the grain that differentiates it from that treated with water (T1) and sugar cane juice (T2). In contrast, some of the T4 and T3 samples clustered differently (Figure 5). However, this behavior could not be considered anomalous, according to the analysis of the residual sums of squares (RSS) and Hotelling's T2 (T2) because they are not strong extremes that generate considerable changes in the analysis of information (Figure 6A and B).

Thus, the chemical composition of coffee beans is affected by the addition of sugarcane juice and yeast during wet fermentation. This effect can be considered favorable when related to the sensory results obtained from the T2, T3, and T4 samples that showed better scores in the SCA sensory analysis with 82.87±2.03, 83.31±2.29, and 81.30±2.34, respectively, compared to the control treatment (T1) that obtained a score of 73.88±5.27. In addition, it was evident that treatments (T2 and T3) with the best sensory results (Table 2) clustered distantly from the treatment with the lowest sensory quality (T1) (Figure 5).

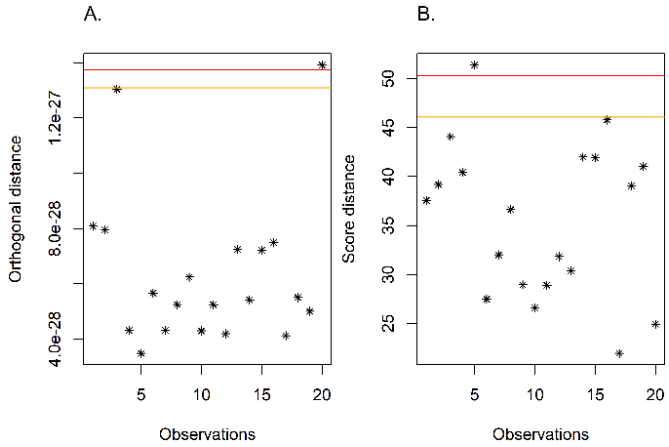


Figure 6. Analysis of residual sums of squares (RSS) (A) and Hotelling's T2 (B). (Red line percentile 99% and yellow line percentile 95%)

Conclusions

1. Adding sugar cane juice or the combination of sugar cane juice with *Saccharomyces cerevisiae* for the coffee Colombia variety, improved the beverage quality, evidencing scores in the range of 81.30 to 83.31 according to the SCA scale, while the control samples were classified as non-specialty coffee (73.88 points).

2. Chemometric analysis of the spectral data corroborated that the addition of sugarcane juice and yeast affected the chemical composition of the coffee beans.

3. The addition of cane juice only (T2) and of the Oenoferm® Freddo yeast strain (T3) presented the best sensory quality.

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