**Review article**

# Coffee cherry Fermentation by *Saccharomyces cerevisiae* var. *bayanus* for beverage and snack development

# Fermentación de café cereza con *Saccharomyces cerevisiae* var. *bayanus* para el desarrollo de bebida y snack

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**ABSTRACT**
The valorization of coffee processing residues through controlled fermentation represents a sustainable alternative within a circular economy model. This study aimed to evaluate the potential of *Saccharomyces cerevisiae* var. *bayanus* as a fermenting agent of coffee cherries for the development of a fermented beverage and the assessment of coffee as a potential snack. A comparative study was conducted with two treatments established according to substrate composition: T (coffee cherries and water) and M (coffee cherries, water, and honey), under controlled temperature and time conditions. Physicochemical and microbiological variations were recorded during fermentation. Treatment M showed higher soluble solids, acidity, and alcohol content compared with T, while viscosity was similar between treatments. In the sensory evaluation, coffee brewed from treatment T obtained a higher score than M, revealing differences attributable to substrate composition. Fermentation with *Saccharomyces cerevisiae* var. *bayanus* demonstrated its feasibility for obtaining food by-products with potential consumer acceptance. These results highlight the applicability of directed fermentation as a biotechnological strategy for the valorization of coffee cherries, contributing to the diversification of coffee-derived products and the strengthening of circular economy practices in the coffee sector.

**KEYWORDS:** Fermented products, Food processing, Yeasts, Lactic acid bacteria

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**RESUMEN**
La valorización de los residuos del procesamiento del café mediante fermentación controlada representa una alternativa sostenible dentro de un modelo de economía circular. Este estudio tuvo como objetivo evaluar el potencial de *Saccharomyces cerevisiae* var. *bayanus* como agente fermentador de café cereza para el desarrollo de una bebida fermentada y el análisis del café como posible snack. Se realizó un estudio comparativo con dos tratamientos establecidos según la composición del sustrato: T (café cereza y agua) y M (café cereza, agua y miel), manteniendo condiciones controladas de temperatura y tiempo. Durante la fermentación se registraron variaciones fisicoquímicas y microbiológicas. El tratamiento M presentó mayores valores de sólidos solubles, acidez y contenido alcohólico en comparación con T, mientras que la viscosidad fue similar entre tratamientos. En la evaluación sensorial, el café en taza del tratamiento T obtuvo un puntaje superior al de M, evidenciando diferencias atribuibles a la composición del sustrato. La fermentación con *Saccharomyces cerevisiae* var. *bayanus* demostró su viabilidad para la obtención de subproductos alimentarios con potencial de aceptación en consumidores. Estos resultados destacaron la aplicabilidad de la fermentación dirigida como una estrategia biotecnológica para valorizar el café cereza,

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contribuyendo a la diversificación de productos derivados del café y al fortalecimiento de prácticas de economía circular en el sector cafetero.

**PALABRAS CLAVE:** Productos fermentados, Procesamiento de alimentos, Levaduras, Bacterias ácido-lácticas

## INTRODUCTION

Colombia is the third largest coffee producer worldwide, with approximately 654,000 farms and 540,000 growers (Montoya et al. 2023). Within this context, the department of Huila plays a strategic role in the national economy, as coffee represents both the main agricultural export product and the largest source of rural employment (Cerquera et al. 2020). One of the major challenges of the sector is the management of coffee wastes, which, when discarded without proper treatment, generate significant environmental pollution in coffee-growing regions. In this regard, the circular economy model offers an opportunity to transform these residues into valuable inputs for new production chains for example, using coffee pulp as fertilizer, feedstock for bioenergy, or substrate for fermentation processes (Geissdoerfer et al. 2020).

Recent advances in coffee biotechnology have focused on the use of controlled or "directed" fermentation to modulate sensory quality and valorize coffee residues. This approach involves the inoculation of specific microbial strains to standardize metabolic pathways and enhance the development of desirable volatile and non-volatile compounds (de Melo Pereira et al. 2022). *Saccharomyces cerevisiae* is widely recognized for its role in food fermentations; however, certain strains such as *Saccharomyces cerevisiae* var. *bayanus*, commonly used in winemaking, possess distinctive metabolic capabilities particularly in sugar tolerance and ester formation that remain underexplored in coffee matrices (dos Santos Silva et al. 2024). This scientific gap limits the understanding of how non-traditional yeasts could improve the biochemical complexity and functional potential of fermented coffee-based products.

In this framework, honey is recognized as a biological resource of interest for sustainable production models due to its complex composition, dominated by carbohydrates and enriched with bioactive compounds, organic acids, minerals, and volatile substances (Campo and Hincapié 2023). Furthermore, its potential as an environmental biomarker enables the assessment of soil, water, and air contamination, thereby expanding its functional and strategic relevance within agri-food chains.

Therefore, the aim of this work was to study the directed fermentation of coffee cherries inoculated with *Saccharomyces cerevisiae* var. *bayanus,* evaluating its effect on the physicochemical and sensory characteristics of the resulting beverage and assessing the feasibility of valorizing both pulp and beans for the development of novel coffee-derived snacks.

## MATERIALS AND METHODS

### Raw material collection

A representative sample of 12 kg of coffee cherries, corresponding to the Caturra and Castillo varieties (6 kg per variety), was collected from the Criollo village, located in the southern region of Huila department, Pitalito municipality, at an approximate altitude of 1,318 meters above sea level (masl). During collection, only fully ripe fruits were selected to ensure sample homogeneity. The cherries were transported to the pilot plant of the South Colombian Coffee Research Center – CESURCAFE, using expanded polystyrene containers with cooling gels to maintain a low temperature and prevent premature initiation of fermentation, thus preserving the physicochemical and microbiological integrity of the fruits.

### Fruit selection and juice extraction

The collected samples were processed at the CESURCAFÉ pilot coffee processing plant, Faculty of Engineering, Universidad Surcolombiana, Neiva. A GAVIOTA 300 pulper (capacity 300 kg $h^{-1}$) equipped

with a cylindrical sieve on rods for grain classification was used, in accordance with Colombian Technical Standard 2090 (NTC 2090).

Once the pulp was separated from the seeds, all components (pulp, seeds, and mucilage) were deposited together in four 8 L plastic containers. These containers were equipped with a bottom valve and a lid adaptation consisting of an air trap, to which sterile water was added to prevent oxygen entry and facilitate $CO_2$ release during alcoholic fermentation, ensuring a controlled anaerobic environment.

For the preparation of the must, a 60:40 water –cherry ratio was used, corresponding to 3 kg of cherries and 4.5 L of potable water per container, reaching a total volume of 7.5 L in each. Each experimental treatment was conducted in duplicate: two controls without honey addition (T1 and T2) and two treatments with honey (M1 and M2), ensuring replicability and consistency.

**Treatments**

The experimental treatments were designed to evaluate the effect of adding bee honey during coffee cherry fermentation. Control treatments (T1 and T2) consisted of 1,340 g of pulp, 1,660 g of seeds, and 4,500 mL of potable water per container, without any sweetener. Treatments with honey (M1 and M2) included the same components as the controls, with the addition of 2,000 g of bee honey (Apisred S.A.S., Colombia).

After homogenously distributing the water – cherry proportions (60:40) in each container, chaptalization was performed only in treatments M1 and M2 by adding honey until reaching 21 °Brix. Treatments T1 and T2 were maintained as controls, allowing comparative evaluation of the effects of honey on alcoholic fermentation, metabolite development, and final beverage characteristics.

**Alcoholic fermentation**

In each container, 1.5 g of *Saccharomyces cerevisiae* var. *bayanus* (Oenoferm Color) yeast was added, previously activated in a portion of the mixture obtained from each tank. For activation, the samples were placed in a water bath (Memmert, Germany) until reaching 30 °C. The activated portion was then inoculated into the corresponding tank at room temperature, ensuring uniform initiation of the fermentation process.

Physicochemical parameters were monitored every 24 hours for 14 days in control treatments (T1 and T2) and for 24 days in honey treatments (M1 and M2), to evaluate fermentation progress. Microbiological monitoring was performed every 24, 48, and 72 hours, as well as at the end of the process, before beverage bottling and after 12 months of maturation, ensuring control of microbial development and product stability.

After 48 hours of fermentation, the solids in each container (pulp and seeds) were removed by filtration, retaining only the liquid fraction (cherry juice). The recovered solids were separated and subjected to additional processes, described in subsequent sections, to maximize the integral use of raw material and evaluate coffee-derived products.

**Washed coffee beans**

The coffee beans obtained after phase separation were sun-dried until reaching a moisture content of 11%, determined using a portable KETT grain moisture tester (PM-450, Santiago Boulevard). Subsequently, the beans were subjected to physical analysis according to standards established by the Federación Nacional de Productores de Café for export coffee, and to sensory analysis following the Specialty Coffee Association (SCA) protocol, to evaluate quality attributes and organoleptic profile.

**Clarification**

In the control treatments (T1 and T2), after 10 days of fermentation, unflavored gelatin (Royal) was added at a ratio of 1 g per liter of coffee cherry juice. After addition, the mixture was carefully homogenized and allowed to rest for 24 hours to promote sedimentation of solids and obtain a clarified liquid.

In contrast, in honey treatments (M1 and M2), gelatin was not used; solids precipitated naturally, and the phases were separated, retaining only the clarified supernatant for subsequent bottling. This procedure ensured stable juice, free of suspended particles, while maintaining the organoleptic and physicochemical properties developed during fermentation.

**Bottling**
The clarified liquid fraction was deposited into amber bottles, previously washed and disinfected, to minimize light exposure and microbial contamination. Bottles were then sealed with corks and properly labeled to ensure correct identification of each treatment and replicate during maturation and subsequent analyses. This procedure ensured product integrity and experimental traceability.

**Maturation**
The fermented coffee cherry beverage was subjected to a maturation process in bottles for 365 days. Bottles were stored horizontally, ensuring the liquid completely covered the cork, minimizing oxygen ingress, and preserving the organoleptic and physicochemical properties developed during fermentation. This procedure provided a controlled environment for the evolution of aromatic compounds and stabilization of the final product.

**Distillation**
A simple distillation setup was used with 250 mL of fermented beverage, previously matured for 30 days. The process was maintained at a constant temperature of 70–80 °C for approximately 4 hours, allowing efficient separation of the alcoholic component from the rest of the liquid matrix.

The alcohol content of the distillate was determined using the Gay-Lussac equation (Equation 1), providing a precise measurement of the ethyl concentration in the final product. This procedure allowed reliable quantification of alcohol produced during fermentation and evaluation of the effects of different treatments on alcohol production.

Alcoholic strength in Gay-Lussac:

$$°GL = (\text{Volume distilled}) * 100 / (\text{Volume drink}) \quad (1)$$

**Determination of physicochemical parameters**
**Soluble Solids (°Brix)**
The soluble solids content (°Brix) for the cherry coffee fermented beverage samples was determined with a digital refractometer (Atago Digital PR-201α) by the refractometer method (AOAC-932.12), placing a small sample of the beverage corresponding to each treatment.

**Hydrogen Potential (pH)**
The pH was determined with the OHAUS Started (5,000) potentiometer, previously calibrated according to the AOAC-981.12 Standard (1997). The value was obtained by inserting the electrode directly into the sample.

**Titratable acidity**
The titratable acidity was established according to the standard (AOAC-942.15). The titratable acidity was determined according to the equivalent of chlorogenic acid, which is the predominant acid in coffee pulp. It is expressed in ($g\ L^{-1}$).

**Viscosity**

Viscosity was measured with the Brookfield DV3T Rheometer. Samples of 10 mL were run at 120 rpm with the ULA needle and an average torque of 35%. The viscosity is calculated according to Equation 2.

$$\text{Viscosity} = \text{shear stress/shear rate} \quad (2)$$

The result is expressed in centipoise (cP), which is equivalent to 1 mPa s (millipascal second).

**Color - CieLab Coordinates**

A KONICA MINOLTA CR-410 HEAD mobile colorimeter was used to measure the parameters of the CIE method (International Commission on Illumination). The measurement was performed with 20 mL of sample of the different treatments, the CIE coordinates L, a, and b were obtained, and the chroma, hue, and ΔE were calculated from them.

Equations 3, 4 and 5 show how to calculate C, H and ΔE, respectively.

Croma (C):

$$C=\sqrt{(a^2+ b^2)} \quad (3)$$

Hue (H):

$$H= \arctan\left(\frac{b}{a}\right) \quad (4)$$

ΔE:

$$\Delta E=\sqrt{(\Delta L^2)+(\Delta a)^2+(\Delta b)^2} \quad (5)$$

**Determination of microbiological parameters**

**Yeast count**

Serial dilutions from $10^{-2}$ to $10^{-10}$ were performed in test tubes with 9 mL of peptone water (Merck KGaA, country). Then, deep seeding was performed with YGC agar (Chloramphenicol Glucose Agar-Conda, Pronadisa KGaA, Spain), in duplicate in a horizontal laminar flow cabinet (Diseño Industrial, Colombia). Subsequently, the plates were incubated at 30 °C in aerobiosis conditions for 48 hours in the incubator (Thermo Scientific Heratherm IMH100, Germany). This procedure was repeated before bottling and after 365 days of maturation.

**Lactic Acid Bacteria Count (BAL)**

After 30 days of in-bottle maturation, serial dilutions were performed in test tubes with 9 mL of MRS broth (Conda-Pronadisa, Spain), then deep seeding was performed in MRS agar (Conda-Pronadisa, Spain), in duplicate in a horizontal laminar flow cabinet (Diseño Industrial, Colombia). The plates were incubated at 37 °C in aerobiosis conditions for 48 hours in the incubator (Thermo Scientific Heratherm, Germany). This procedure was repeated after 365 days of in-bottle maturation. Then, the most representative colonies of yeasts and lactic acid bacteria (LAB) were identified morphologically, detailing their macroscopic and microscopic characteristics. This was achieved by applying simple and Gram staining, respectively, for a more detailed observation.

**Sensory evaluation**

**Sensory evaluation of fermented beverages**

After a year of maturation, the work was socialized with coffee communities in southern Huila, who evaluated the color, cleanliness, aroma, flavor, acidity, alcohol, body, aftertaste and general impression and

rated the beverage using a hedonic test with a scale of 1 to 10, with 1 being the lowest degree of satisfaction and 10 the highest.

**Sensory evaluation of coffee in a cup**

The coffee rating was carried out at CESURCAFE following the SCA and the Colombian Technical Standards 2758 and 3566 (NTC 2758; NTC 3566), by a panel formed by four trained evaluators.

**Sensory evaluation in snacks**

The snack was evaluated at CESURCAFE by a panel of 30 non-expert judges who conducted a hedonic test, evaluating shape, flavor, aroma, and texture on a scale of 1 to 10, with 1 being the lowest degree of satisfaction and 10 the highest.

**Statistical analysis**

The results obtained were analyzed using an analysis of variance (simple and multifactorial ANOVA), with a confidence level of 95%, establishing whether there are statistically significant differences in the variables evaluated depending on the treatments evaluated. The Statgraphics Centurion XVI Version 16.1.03 software was used.

## RESULTS AND DISCUSSION

### Physicochemical, microbiological and sensory analysis of fermentation processes between treatments

**Physicochemical Analysis**

Tables 1 and 2 show the mean values and standard deviations of the physicochemical parameters quantified in treatments T and M, respectively.

**Table 1**. Mean values and standard deviations of physicochemical parameters quantified in the T treatment.

| Time (Days) | Soluble Solids (°Brix) | Hydrogen Potential (pH) | Acidity (g $L^{-1}$) | Viscosity (Cp) |
|---|---|---|---|---|
| 0 | 3.35±0.05$^{efg}$ | 5.28±0.03$^{g}$ | 1.07±0.02$^{a}$ | 1.73±0.08$^{b}$ |
| 1 | 3.93±0.08$^{h}$ | 4.45±0.09$^{f}$ | 1.41±0.03$^{b}$ | 3.22±0.52$^{d}$ |
| 2 | 3.73±0.08$^{gh}$ | 3.80±0.09$^{e}$ | 1.62±0.02$^{c}$ | 1.20±0.02$^{a}$ |
| 3 | 3.70±0.11$^{gh}$ | 3.75±0.06$^{e}$ | 1.67±0.04$^{cd}$ | 2.01±0.46$^{c}$ |
| 4 | 3.58±0.13$^{fg}$ | 3.61±0.13$^{cd}$ | 1.65±0.05$^{c}$ | 1.61±0.01$^{b}$ |
| 5 | 3.48±0.20$^{efg}$ | 3.63±0.04$^{cd}$ | 1.71±0.03$^{de}$ | 1.59±0.01$^{b}$ |
| 6 | 3.20±0.62$^{cde}$ | 3.63±0.03$^{cd}$ | 1.73±0.03$^{ef}$ | 1.57±0.01$^{b}$ |
| 7 | 3.08±0.53$^{bcd}$ | 3.56±0.03$^{c}$ | 1.77±0.00$^{fg}$ | 1.58±0.01$^{b}$ |
| 8 | 2.98±0.46$^{abc}$ | 3.61±0.05$^{cd}$ | 1.77±0.00$^{fg}$ | 1.57±0.02$^{b}$ |
| 9 | 2.68±0.31$^{a}$ | 3.61±0.05$^{cd}$ | 1.77±0.00$^{fg}$ | 1.56±0.02$^{b}$ |
| 10 | 2.85±0.27$^{ab}$ | 3.64±0.03$^{d}$ | 1.79±0.02$^{g}$ | 1.57±0.01$^{b}$ |
| 11 | 3.00±0.22$^{abc}$ | 3.33±0.08$^{b}$ | 1.89±0.02$^{h}$ | 2.23±0.09$^{c}$ |
| 12 | 3.00±0.22$^{abc}$ | 3.28±0.04$^{ab}$ | 1.89±0.02$^{h}$ | 2.19±0.09$^{c}$ |
| 13 | 2.90±0.11$^{abc}$ | 3.21±0.07$^{a}$ | 1.97±0.08$^{i}$ | 1.67±0.01$^{b}$ |
| 14 | 2.87±0.12$^{abc}$ | 3.21±0.07$^{b}$ | 2.01±0.02$^{i}$ | 1.60±0.01$^{b}$ |

S.D.: Standard Deviation; a, b, c, d, e, f, g, h, i, j, k. Different letters in the same column indicate statistically significant changes from each other ($P \leq 0.05$); $\tilde{x}$±S.

**Table 2.** Mean values and standard deviations of physicochemical parameters quantified in the M treatment.

| Time (Days) | Soluble Solids (°Brix) | Hydrogen Potential (pH) | Acidity (g $L^{-1}$) | Viscosity (Cp) |
|---|---|---|---|---|
| 0 | 21.53±0.19$^{u}$ | 4.46±0.02$^{o}$ | 2.06±0.00$^{a}$ | 3.64±0.08$^{n}$ |
| 1 | 20.82±0.15$^{st}$ | 4.05±0.04$^{n}$ | 2.53±0.04$^{b}$ | 4.46±0.08$^{i}$ |
| 2 | 20.88±0.08$^{t}$ | 3.65±0.01$^{m}$ | 3.41±0.02$^{c}$ | 1.78±0.03$^{d}$ |
| 3 | 20.70±0.00$^{s}$ | 3.57±0.02$^{l}$ | 3.57±0.02$^{d}$ | 1.91±0.01$^{ef}$ |
| 4 | 20.50±0.17$^{r}$ | 3.46±0.01$^{k}$ | 3.65±0.00$^{d}$ | 1.95±0.02$^{g}$ |
| 5 | 20.33±0.08$^{q}$ | 3.40±0.01$^{j}$ | 3.76±0.15$^{e}$ | 1.94±0.02$^{fg}$ |
| 6 | 19.05±0.23$^{p}$ | 3.37±0.00$^{ij}$ | 3.90±0.00$^{f}$ | 1.96±0.03$^{g}$ |
| 7 | 17.87±0.12$^{o}$ | 3.28±0.01$^{gh}$ | 3.90±0.00$^{f}$ | 1.90±0.02$^{e}$ |
| 8 | 15.92±0.15$^{n}$ | 3.33±0.01$^{hi}$ | 3.90±0.00$^{f}$ | 1.78±0.02$^{d}$ |
| 9 | 13.43±0.05$^{m}$ | 3.33±0.01$^{hi}$ | 3.90±0.00$^{f}$ | 1.68±0.01$^{c}$ |
| 10 | 12.35±0.19$^{l}$ | 3.35±0.00$^{ij}$ | 3.92±0.02$^{f}$ | 1.68±0.02$^{c}$ |
| 11 | 11.17±0.26$^{k}$ | 3.26±0.05$^{fg}$ | 4.02±0.02$^{g}$ | 1.67±0.01$^{c}$ |
| 12 | 10.30±0.11$^{j}$ | 3.21±0.07$^{ef}$ | 4.03±0.02$^{g}$ | 1.66±0.01$^{c}$ |
| 13 | 9.90±0.18$^{i}$ | 3.20±0.08$^{e}$ | 4.07±0.03$^{g}$ | 1.65±0.01$^{c}$ |
| 14 | 9.35±0.16$^{h}$ | 3.18±0.07$^{e}$ | 4.08±0.03$^{g}$ | 1.61±0.03$^{ab}$ |
| 15 | 9.20±0.06$^{g}$ | 3.11±0.13$^{d}$ | 4.21±0.17$^{h}$ | 1.60±0.01$^{a}$ |
| 16 | 8.87±0.05$^{f}$ | 3.10±0.08$^{cd}$ | 4.26±0.11$^{h}$ | 1.60±0.01$^{a}$ |
| 17 | 8.50±0.06$^{e}$ | 3.06±0.08$^{cd}$ | 4.27±0.10$^{h}$ | 1.59±0.01$^{b}$ |
| 18 | 8.42±0.08$^{de}$ | 3.05±0.05$^{c}$ | 4.27±0.10$^{h}$ | 1.59±0.02$^{a}$ |
| 19 | 8.35±0.05$^{cd}$ | 2.96±0.05$^{b}$ | 4.42±0.07$^{i}$ | 1.57±0.02$^{a}$ |
| 20 | 8.25±0.05$^{bc}$ | 2.93±0.05$^{ab}$ | 4.42±0.08$^{i}$ | 1.57±0.01$^{a}$ |
| 21 | 8.15±0.05$^{b}$ | 2.95±0.05$^{ab}$ | 4.42±0.08$^{i}$ | 1.57±0.01$^{a}$ |
| 22 | 8.00±0.00$^{a}$ | 2.95±0.00$^{ab}$ | 4.42±0.08$^{i}$ | 1.56±0.01$^{ab}$ |
| 23 | 8.00±0.00$^{a}$ | 2.90±0.00$^{a}$ | 4.50±0.00$^{i}$ | 1.56±0.00$^{ab}$ |
| 24 | 8.00±0.00$^{a}$ | 2.90±0.00$^{a}$ | 4.50± 0.00$^{i}$ | 1.56±0.01$^{ab}$ |

S.D.: Standard Deviation; a, b, c, d, e, f, g, h, i, j, k, l, m, n, o, p, q, r. Different letters in the same column indicate statistically significant changes from each other ($P \leq 0.05$); x̃±S.

**Soluble Solids**

The consumption of sugars by yeast populations is a determining factor in ethanol production and directly reflects the metabolic efficiency of the fermentation process (Sholichah et al. 2021). In treatment M, the addition of honey increased the initial concentration of soluble sugars to 21.53 °Brix, which subsequently decreased to 8.00 °Brix by the end of fermentation. This substantial reduction demonstrates a high metabolic activity of *Saccharomyces cerevisiae* var. *bayanus* and effective conversion of fermentable sugars into ethanol and secondary metabolites (Triviño et al. 2021).

In contrast, the control treatment (T) exhibited only a slight decrease from 3.35 to 2.87 °Brix, indicating limited substrate availability and, consequently, lower yeast activity. The stabilization of soluble solids values after fermentation suggests the near depletion of fermentable carbohydrates and the establishment of metabolic equilibrium within the medium (Dorta et al. 2024). Statistically significant differences were observed between both treatments at the beginning and the end of the process, confirming the influence of substrate composition on the fermentative performance of the yeast.

**Hydrogen Potential**

The pH values in treatments T and M remained within an acidic range both before and after the fermentation process, with initial values of 5.28 and 4.46 and final values of 3.21 and 2.90, respectively. These variations

denote statistically significant differences between the beginning and the end of fermentation, evidencing the acidification process typical of yeast-driven fermentations. The progressive decline in pH can be attributed to the accumulation of organic acids formed through the metabolism of fermentable sugars and the enzymatic degradation of pectin present in the coffee mucilage (Peñuela et al. 2021).

The control treatment (T) exhibited a more pronounced pH drop compared to M, which may be related to the lower buffering capacity of the medium without honey addition. Conversely, in M, the presence of honey provided additional substrates that favored a balanced acid production, maintaining a slightly higher final pH.

The pH plays a fundamental role in coffee mucilage fermentation, as it directly influences microbial activity, enzymatic stability, and the quality of the final beverage. According to Osorio et al. (2022), the pH of coffee mucilage typically decreases from around 5.3 to near 3.2 as fermentation progresses. This trend, consistent with the present study, indicates the increasing production of acids derived from microbial metabolism. Therefore, pH monitoring can serve as a reliable indicator for determining the optimal endpoint of fermentation, ensuring both microbial control and desirable sensory characteristics in the resulting product.

**Titratable Acidity**

The evolution of titratable acidity during fermentation revealed clear differences between treatments. In treatment T, the final concentration reached 2.01 g $L^{-1}$, whereas in treatment M, acidity continued to increase beyond day 14, attaining a final value of 4.50 g $L^{-1}$. During the first two days of fermentation, both treatments exhibited a marked rise in acidity, followed by a slower but steady increase in subsequent stages. This trend reflects the catabolic processes associated with microbial metabolism, membrane degradation, and the biochemical activity of compounds naturally present in the coffee cherry, all of which contribute to acid accumulation in the medium (Amorocho-Cruz and Muñoz-Cortés 2021).

According to Galarza and Figueroa (2022), the degradation of pectin and simple sugars in the mucilage enhances the release of organic acids such as citric, lactic, and acetic acids, intensifying the overall acidity of the substrate. This increase not only characterizes the progress of fermentation but also plays a crucial role in shaping the sensory attributes of the final product, as a balanced acidity contributes to the perception of freshness, complexity, and aromatic definition in coffee-based beverages.

Statistical analysis confirmed significant differences between treatments at both the beginning and the end of the fermentation process, underscoring the influence of substrate composition particularly the presence of honey on acid production dynamics (Anwar et al. 2025).

**Viscosity**

During the first 24 hours of fermentation, both treatments exhibited a notable increase in viscosity, which can be attributed to the high initial concentration of sugars that promoted intense microbial activity and the synthesis of polysaccharides and extracellular compounds, as described by Mengi et al. (2020). This stage corresponds to the exponential phase of yeast growth, during which the metabolic transformation of carbohydrates results in transient thickening of the medium.

Subsequently, a progressive decrease in viscosity was recorded, followed by stabilization in treatment M, which is associated with sugar degradation, ethanol formation, and a reduction in microbial viability toward the end of fermentation. In treatment T, although a decrease was observed between day 1 and 14, it was not statistically significant. Conversely, treatment M showed a reduction of approximately two viscosity units, accompanied by statistically significant differences, suggesting a more dynamic fermentative process influenced by the presence of honey as an additional carbon source.

On day 10, neutral food gelatin was added to treatment T for clarification purposes; however, an unexpected increase in viscosity was observed the following day. This effect was attributed to excessive gelatin binding, which hindered flocculation and sedimentation of suspended particles, leading to turbidity in the medium. Consequently, treatment T was discontinued. In contrast, treatment M did not employ gelatin as a clarifying agent; instead, the beverage was filtered through filter paper before bottling, ensuring a clearer final product without interfering with the physical stability of the matrix.

**CIELab coordinates**

The colorimetric analysis of the fermented beverages based on the CIELab system revealed perceptible visual differences between the two treatments (Table 3). The calculated Delta E (ΔE) values confirmed that the samples were distinguishable to the human eye, indicating that the fermentation substrates produced measurable variations in color attributes.

The Chroma (C) values reflected the intensity of color saturation, evidencing strong chromatic stimuli associated with the pigments present in the coffee pulp and their transformation during fermentation. Such variations are likely related to the oxidation of phenolic compounds and the formation of Maillard reaction products, which contribute to the generation of brownish and amber tones characteristic of fermented coffee matrices (Tan Y et al. 2023).

The Hue angle (H) ranged between 47° and 78°, suggesting an orange hue typical of intermediate stages of pigment oxidation. These results collectively indicate that both treatments underwent distinct chromatic evolution, influenced by the composition of the fermentation substrate and the metabolic activity of *Saccharomyces cerevisiae* var. *bayanus*, which may alter pigment stability and visual properties of the final product (Koren D et al. 2020).

**Table 3.** Average final values and standard deviations of the parameters calculated from the CIELab coordinates.

| Treatment | ΔE | Croma (C) | Hue (H) |
|---|---|---|---|
| T | 28.93±1.03 | 27.25±2.38 | 47.74±0.51 |
| M | 36.01±0.14 | 52.13±0.21 | 78.37±0.69 |

**Determination of alcohol content**

Two 250 mL sub-samples of treatment M (designated M1 and M2) were distilled, yielding 22.4 mL of alcohol in each case, corresponding to approximately 8.96 °GL (Gay-Lussac degrees). This result reflects the metabolic efficiency of the fermenting microorganisms, primarily yeasts, which transform the fermentable sugars present in the coffee cherries and honey into ethanol and other volatile metabolites through glycolytic and fermentative pathways (Stanzer et al. 2023).

**Microbiological Analysis**

The metabolic activity of the yeasts was evaluated during the first 72 hours, obtaining the behavior expressed in $Log_{10}$ CFU $mL^{-1}$ over time as illustrated in Figure 1. The fermentation process was developed with a yeast population of $10^{-7}$ CFU $mL^{-1}$, which remained constant throughout the process.

At the end of the fermentation period for treatment M (day 24), an additional microbiological analysis revealed the complete absence of yeasts and lactic acid bacteria (LAB). This observation can be attributed to the inhibitory effects of accumulated ethanol, which can suppress or eliminate microbial viability once alcohol concentrations exceed tolerance thresholds. Previous studies have demonstrated that honey contains various antimicrobial compounds, including hydrogen peroxide and phenolic substances that inhibit microbial proliferation in fermentative systems (Osés et al. 2024; Brudzynski 2021).

Souza et al. (2024) reported comparable behavior when developing a mead with probiotic potential, in which the final product exhibited stable physicochemical parameters, pH=3.48 and 4.77% (v/v) alcohol, that promoted product preservation while limiting microbial proliferation. By analogy, the high alcohol content and acidic pH reached in treatment M likely contributed to the decline and eventual absence of viable microorganisms at the end of the process, reinforcing the relationship between physicochemical evolution and microbial stability in fermented systems.

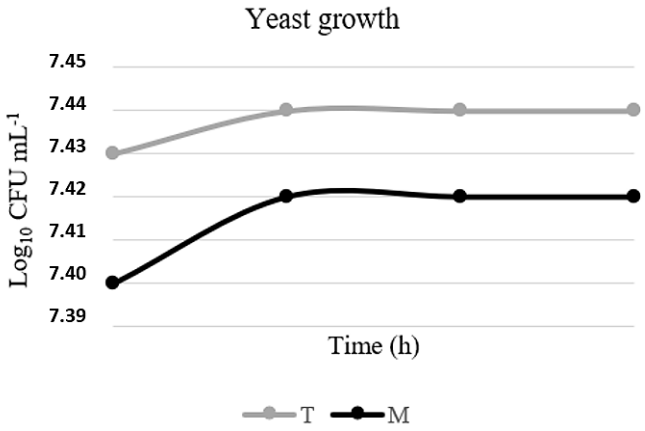


**Figure 1.** Yeast growth represented in $Log_{10}$ CFU $mL^{-1}$ with respect to time.

**Sensory evaluation**

**Sensory evaluation of fermented beverage**

The sensory evaluation of the fermented beverage revealed no statistically significant differences among the samples, as the processing and fermentation conditions were kept constant throughout the experimental period. Overall, the panelists assigned high scores to all evaluated attributes, with values equal to or greater than six on the hedonic scale (Figure 2).

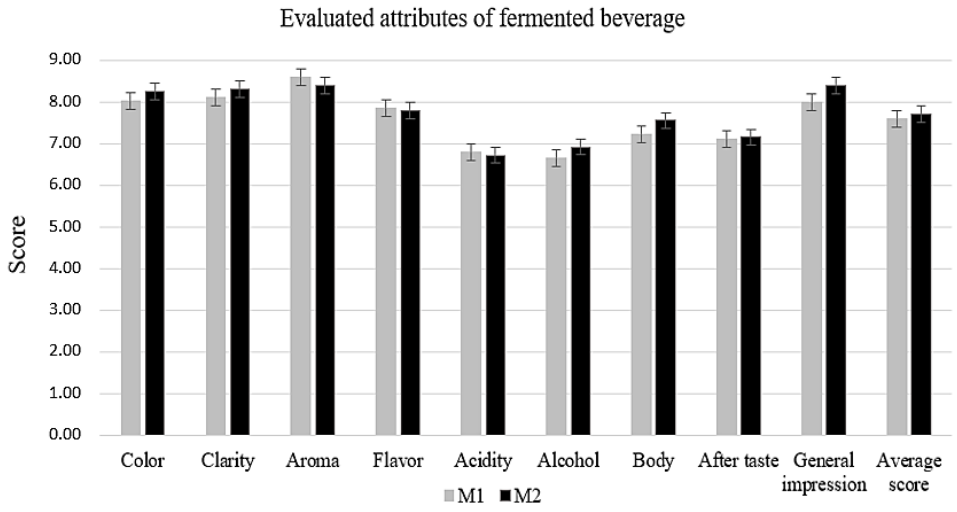


**Figure 2.** Behavior of the attributes evaluated in the fermented beverage for each treatment.

Aroma was identified as the most appreciated sensory attribute, closely associated with the characteristic fragrance of coffee developed during fermentation and subsequent processing. This positive perception is consistent with the volatile profile typically generated by *Saccharomyces cerevisiae*, which contributes to the formation of esters and higher alcohols that enhance the aromatic complexity of the beverage.

In contrast, the attributes related to acidity and alcohol content received comparatively lower ratings. This response can be explained by the synergistic effect of low pH and elevated ethanol concentrations, which may produce an intense astringency and sharp mouthfeel perceived as unpleasant by some tasters. Similar effects have been reported in other fermented beverages, where ethanol and organic acids significantly influence sourness and astringency perception (de la Fuente-Blanco et al. 2024; Thibodeau and Pickering 2021). Nonetheless, the overall acceptance levels suggest that the product retained a desirable sensory balance within the evaluated range.

**Sensory evaluation of coffee in cup**

Figure 3 shows the sensory analysis of brewed coffee revealed that treatment T achieved a total score of 82.75, outperforming treatment M, which obtained 76.63 points. According to the Specialty Coffee Association (SCA) standards, this difference reflects the influence of substrate composition on flavor development. The addition of honey in treatment M introduced distinct aromatic and chemical compounds that modified flavor intensity and balance, leading to perceptible variations in the sensory profile compared with the control treatment.

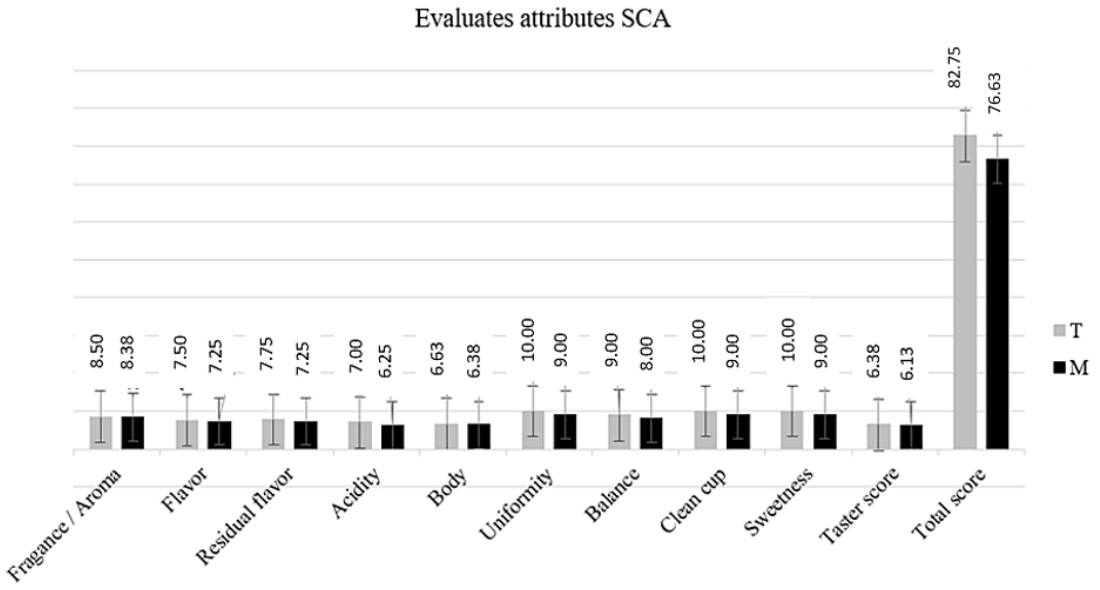


**Figure 3**. Attributes evaluated in coffee cupping according to the SCA.

Among the evaluated attributes, uniformity, clean cup, and sweetness achieved the highest ratings, with scores of 10.0 and 9.0 for treatments T and M, respectively, values considered outstanding under SCA guidelines. Conversely, acidity, body, and taster score showed comparatively lower evaluations, ranging between 6.25 and 7.0 points, corresponding to the "good" category. Intermediate attributes such as aroma, flavor, aftertaste, and balance obtained values between 7.25 and 9.0, classifying them as "very good" to "excellent."

Overall, the sensory performance of the control sample (T) placed it in the "very good" category according to the SCA scale, whereas treatment M did not reach the threshold to be classified as specialty coffee. This difference suggests that substrate composition, particularly the addition of honey, may alter the formation

of aromatic and volatile compounds, affecting the sensory balance and overall quality rating of the product. These findings indicate that substrate composition, particularly the inclusion of honey, can alter the sensory matrix of the coffee beverage, affecting both flavor perception and overall quality rating, as also observed in studies on the influence of alternative fermentation substrates on coffee cup attributes (Polanía et al. 2024).

**Sensory evaluation in snacks**

A chocolate couverture mixture filled with oven-dried coffee pulp was prepared, and the parameters shown in Figure 4 were evaluated by 30 non-expert panelists. Regarding color, shape, and aroma, both treatments received similar ratings (6.0 to 6.9), indicating a neutral perception according to the hedonic scale used.

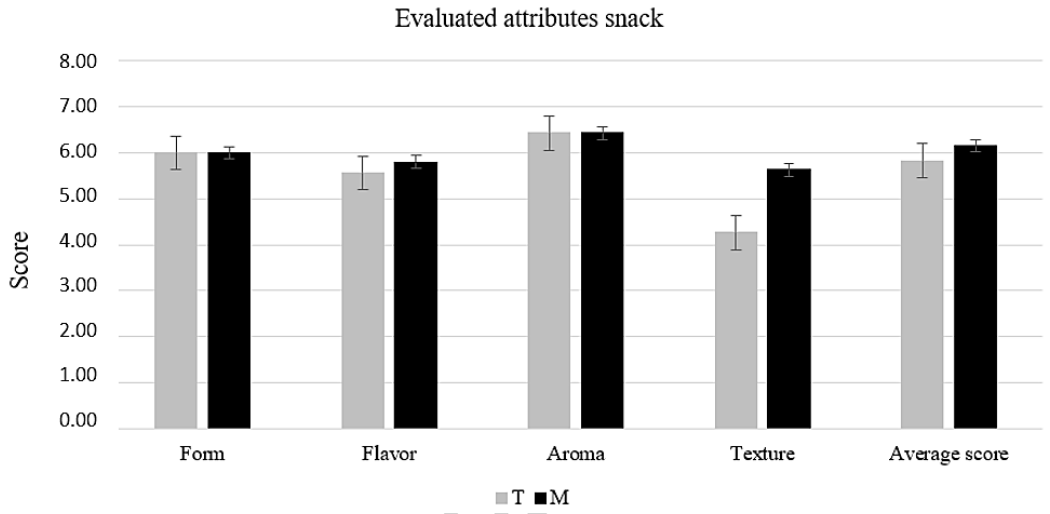


**Figure 4**. Evaluated attributes snack.

In contrast, significant differences were observed in flavor and texture attributes, with the M treatment showing higher scores due to the presence of honey, which enhanced sweetness and smoothness. Despite this improvement, the mean values (4.27 to 5.80) still reflect a moderate level of dissatisfaction among tasters, resulting in overall scores of 5.8 for T and 6.1 for M.

These findings are consistent with previous reports where products incorporating coffee by-products showed limited consumer acceptance when unconventional ingredients altered expected sensory profiles. However, the inclusion of honey as a natural sweetener improved flavor balance and palatability, aligning with studies on functional foods formulated with coffee pulp as a sustainable ingredient source (Pérez Calvo et al. 2023). This suggests that further optimization of formulation, particularly sweetness and texture could enhance consumer acceptance and increase the potential of coffee-derived snacks within circular economy models.

## CONCLUSION

The fermentation of coffee cherries with *Saccharomyces cerevisiae* var. *bayanus* produced a beverage with differentiated physicochemical and sensory characteristics. While treatment T exhibited favorable results in acidity and flavor profile, treatment M showed less desirable sensory attributes, indicating that substrate composition significantly influences the outcome of the fermentation process. These results align with the study's aim of enhancing the valorization of coffee by-products through biotechnological processes that promote circular economy principles within the coffee sector.

The snack formulated from coffee pulp exhibited limited sensory acceptance under current formulation conditions, mainly due to texture and flavor deficiencies, highlighting opportunities for improvement.

Future studies should focus on optimizing fermentation parameters, improving the sensory development of the snack, and evaluating the microbiological stability and shelf life of the products to strengthen sustainable alternatives for coffee by-products utilization.

## CONFLICT OF INTERESTS

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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