Food Biophysics (2026) 21:80
https://doi.org/10.1007/s11483-026-10133-9

RESEARCH

Evaluation of Controlled Fermentation of Coffee Cherries *(Coffea arabica)* with Encapsulated *Meyerozyma caribbica* Inoculation through Dry Processing and its Effect on the Quality

Valentina Escobar Ramírez[1] · Juan David Torres Moreno[1] · Cristina Ramírez-Toro[1,2] · Germán Bolívar[1] · José Luis Plaza-Dorado[2]

Received: 27 October 2025 / Accepted: 30 March 2026 / Published online: 20 June 2026
© The Author(s) 2026

Abstract
Starter cultures represent a viable approach for improving coffee quality through controlled fermentation. This study evaluated the influence of microencapsulated *Meyerozyma caribbica* on dry fermentation of Castillo coffee using a factorial design with two fermentation times (48 and 72 h) and three inoculum concentrations (3%, 5%, and 8%), alongside a control. Physicochemical and microbiological analyses revealed interactions between fermentation time and inoculum concentration for key variables associated with sugar degradation, acidity formation, and microbial dynamics. The scale-up treatment (5%, 48 h) reached 84.50 points according to the Specialty Coffee Association and presented red fruit notes with subtle wine nuances. This treatment also showed phenolic content of 2228.6 mg GAE/100 g and antioxidant capacity of 4072.5 mg Trolox/100 g (ABTS) and 1939.2 mg Trolox/100 g (DPPH). The findings indicate that microencapsulated yeasts contribute to consistent fermentation behavior, improved cup quality, and enhanced functional attributes, offering a practical strategy for specialty coffee production.

Keywords Coffee fermentation · Dry processing · Microencapsulation · *Meyerozyma caribbica* · Biocontrol · Sensory quality

Introduction

Coffee is among the most consumed non-alcoholic beverages worldwide [1, 2]. In Colombia, coffee represents a strategic economic sector, with the 2023–24 harvest reaching 13.9 million 60-kg bags, of which 12.33 million were exported, generating over USD 2.9 billion. This activity supports nearly 550,000 rural families and contributes substantially to national GDP [3, 4].

Despite decades of expertise, Colombian coffee production still faces limitations in postharvest practices, particularly fermentation, washing, and drying. The lack of standardized protocols increases variability, generating physical and sensory defects that restrict access to specialty markets and reduce profitability [5]. Training smallholder producers in standardized methods is therefore essential to improve quality, reduce market risks, and strengthen value chains [6, 7].

Production is dominated by Coffea arabica and wet processing, which involves flotation, depulping, mucilage fermentation, washing, and drying to 10–12% moisture [8, 9]. In contrast, dry processing, predominantly aerobic, favors the retention of glucose and fructose and the proliferation of epiphytic microbial communities supported by pectins and polysaccharide-degrading enzymes [10]. Drying conditions strongly influence volatile compound formation and sensory quality; deviations in moisture promote either structural loss or fungal growth [11].

Although considered slower and less sophisticated, dry processing can yield premium coffees with distinctive profiles and higher market value [12]. Yeast inoculation has

✉ Cristina Ramírez-Toro
cristina.ramirez@correounivalle.edu.co

[1] MIBIA Research Group, Faculty of Natural and Exact Sciences, Marine Biology, Universidad del Valle, Santiago de Cali, Colombia

[2] GIPAB Research Group, School of Food Engineering, Universidad del Valle, Santiago de Cali, Colombia

been shown to enhance sensory traits such as caramel, dried fruits, and vanilla [13], while controlled fermentation influences acidity, flavor, and aroma [14, 15]. The Specialty Coffee Association defines specialty coffee as one free of defects and with a score above 80 points, assessed by certified Q-graders through attributes including fragrance, acidity, balance, and sweetness [16]. In addition, specialty coffees must comply with traceability and sustainability requirements [17].

Recent studies report that yeasts contribute to process control in coffee fermentation by shaping microbial succession and metabolite formation under wet and dry processing conditions, with documented impacts on process consistency and cup quality [18–22]. In wet processing, yeasts contribute to mucilage transformation, while dry processing can benefit from starter inoculation through improved reproducibility under matrix conditions that vary in moisture and solute distribution [18, 22]. Recent reports also describe yeast-mediated suppression of ochratoxin-related fungal risk during postharvest processing through ecological competition and inhibitory metabolites, supporting the use of yeasts as biocontrol agents in coffee fermentation systems [23, 24].

Most controlled coffee fermentations reported in the literature rely on non-encapsulated yeasts, including *Saccharomyces cerevisiae* and *Pichia* spp., where inoculation modifies microbial succession and can improve sensory quality [13, 14, 18, 25]. However, free-cell inoculum remain sensitive to the physical constraints of dry processing, including heterogeneous moisture distribution, locally high solute concentrations, and progressive dehydration of the fruit surface and mucilage. These matrix features can reduce early colonization efficiency and increase variability at smallholder scale. Microencapsulation introduces a biophysical control layer that separates cells from immediate environmental stressors during inoculation and early fermentation while enabling rapid release of viable cells within the coffee mass. This protective microenvironment can support handling and dosing, reduce direct exposure to dehydration and osmotic stress, and improve the probability of starter dominance under field-relevant conditions.

Despite increasing evidence that yeasts contribute to process control and biocontrol during coffee fermentation, few studies have examined encapsulated *Meyerozyma caribbica* as a biological tool to restrict undesirable microorganisms while maintaining or improving sensory quality under dry processing conditions. Addressing this gap, the present study aimed to evaluate controlled dry fermentation of coffee using an encapsulated inoculum of *Meyerozyma caribbica* by integrating (i) physicochemical evolution associated with sugar solubilization and acidification, (ii) microbial dynamics relevant to process control and hygiene indicators, (iii) safety-related outcomes linked to ochratoxin risk, and (iv) quality outcomes based on protocol-based sensory grading and complementary functional measures including total phenolic content and antioxidant capacity.

Materials and Methods

The experimental design was organized into three stages. The first stage included (i) preparation of raw material, (ii) microencapsulation and viability testing of the yeast, and (iii) microbiological characterization of cherries. The second stage comprised (iv) implementation of a factorial experimental design combining fermentation time and inoculum concentration, and (v) physicochemical and microbiological analyses. The third stage focused on (vi) scaling of the most promising treatment and (vii) complementary evaluations of phenolic content, antioxidant capacity, and sensory quality.

Preparation of Raw Material

Coffee cherries (*Coffea arabica* var. Colombia) were harvested at 1557 m.a.s.l. in Morales, Cauca (Colombia). Manual selection was performed based on physiological ripeness, discarding unripe, overripe, or visually defective fruits. The selected cherries were washed using potable water to reduce the microbial load adhered to the exocarp.

Microencapsulation of Yeast for Fermentation

The materials used included cassava starch of the waxy variety (Ingredion Colombia S.A.), sodium alginate (containing D–mannuronic and L–guluronic acids, with molecular weight 80,000–120,000 g/mol; Sigma-Aldrich, Steinheim, Germany), calcium chloride (Agenquímicos, Cali, Colombia), and an exopolysaccharide produced by *Weissella confusa* isolated from sugarcane juice [26], deposited in the MIBIA laboratory collection [27]. The starter microorganism consisted of a microencapsulated *Meyerozyma caribbica* isolate preserved in the MIBIA culture collection at Universidad del Valle and originally obtained from coffee fermentation matrices. Detailed information on strain origin, taxonomic identification, and supporting analyses is provided in the Supplementary Material.

Preparation of Microcapsules

Microcapsules were produced following Briceño-Vélez [28]. Yeast strains were activated in a medium containing 22.24 g sugar, 21.53 g soy milk, 19.41 g whey, 6 g agar, and 300 mL water per 15 mL inoculum, followed by incubation

at 35 °C for 20 h. Cultures were centrifuged at 4000 rpm for 10 min, the supernatant was discarded, and the cell pellet was washed with 0.1% peptone water until reaching a suspension of approximately 9 log CFU/g, determined using the McFarland scale [29].

Cassava starch, EPS, and sodium alginate solutions were sterilized in an autoclave (Market Forget STM-EL, USA) at 121 °C for 15 min. The sterile solutions were then mixed with 10 mL of the active cell suspension on a magnetic stirrer (Benchmark, H3760-H, USA). The resulting mixtures were atomized using a spraying system (Discover LN-I with mini compressor Discover TC-06, USA) equipped with a 0.5 mm nozzle and collected in sterile 0.1 M calcium chloride solution as crosslinking agent. After 15 min of gelation, the material was centrifuged at 4000 rpm for 10 min and dried in a convection oven at 40 °C for 3 h. The dry material was milled and stored for subsequent use as a starter inoculum.

Microcapsule Characterization: Microencapsulation Percentage, Viability, and Release Time

Microcapsules were characterized through microencapsulation percentage, viability, release time in coffee matrix, physicochemical properties (water activity, moisture, solubility, wettability), and particle morphology and size documented by SEM.

Microencapsulation percentage was determined from viable counts of released cells relative to viable counts of free cells added during microcapsule production. One gram of microcapsules was mixed with 9 mL of 0.1% peptone water until disintegration using a homogenizer (Minimix, Interscience, France). Viable counts were obtained by plate counting on malt extract agar after incubation at 37.5 °C for 48 h. Microencapsulation percentage was calculated as: PM (%) = $(N/N_0) \times 100$, where N corresponds to viable released cells (log CFU g^{-1} microcapsules) and N_0 corresponds to viable free cells added to the wall mixture (log CFU g^{-1}) [30].

The viability of microencapsulated *M. caribbica* was evaluated by reactivating 1 g of powdered sample in 9 mL of peptone water, followed by vortex mixing. Serial dilutions were prepared up to 10^{-6}. From the last three dilutions, 100 µL aliquots were spread onto malt extract agar plates, which were incubated at 37.5 °C for 48 h (Kryoven incubator).

Release time was determined in coffee matrix using the approach of Criollo et al. [30] with modifications. Microcapsules were added to 50 g of coffee mass. Sampling was performed every 2 min until viable counts reached the target value defined for the inoculum.

Microcapsule morphology was examined by scanning electron microscopy. Dried microcapsules were mounted on aluminum stubs using conductive carbon tape and coated with a conductive metal layer prior to imaging. Micrographs were acquired at multiple magnifications to document surface features and cross-sectional structure.

Microbiological Characterization of Raw Material

Microbiological characterization of coffee cherries was carried out according to Colombian Technical Standards (NTC) 4519, 4516, and 4132 for coliforms, mesophilic bacteria, and fungi, respectively. One gram of cherries was homogenized in a sterile bag with 9 mL of peptone water using a stomacher (HETTICH Rotofix 32), and serial dilutions from 10^{-1} to 10^{-5} were prepared following Samaniego [31]. For mesophilic bacteria counts, dilutions from 10^{-3} to 10^{-5} were inoculated in triplicate onto Plate Count Agar (PCA) using the surface-spread technique with glass beads, followed by incubation at 37 °C for 48 h; colonies were counted on plates containing 30–300 colonies, and results were expressed as CFU/g [31]. Yeast and mold counts were determined by spreading 0.1 mL aliquots from dilutions 10^{-3} to 10^{-5} onto Potato Dextrose Agar (PDA) in triplicate, with incubation at 27 °C for 72 h (Kryoven incubator), and results were expressed as CFU/g [31]. Presumptive tests for total coliforms were performed by inoculating dilutions 10^{-1} to 10^{-3} into lauryl sulfate broth with Durham tubes (three per dilution), incubating at 37 °C for 48 h, and recording gas production to determine the most probable number (MPN) using the standard table. Confirmatory tests were carried out by streaking gas-positive tubes onto eosin methylene blue (EMB) agar and adding Kovacs reagent to detect indole production; the formation of a red ring indicated presumptive positive results for *E. coli*, while the absence of color change was interpreted as negative. Colonies with a metallic green sheen on EMB were considered positive.

Experimental Design

A 2 × 4 factorial design was implemented with two factors: inoculum concentration (0, 3, 5, and 8% w/w) and fermentation time (48 and 72 h). Each treatment was conducted in triplicate ($n = 3$). Before performing ANOVA, the assumptions of normality and homogeneity of variances were verified using and, respectively. Data were then analyzed by ANOVA with a significance threshold of $p \leq 0.05$, followed by Tukey's multiple-comparison test at a 95% confidence level when significant effects were detected. Statistical analyses were conducted using Minitab 19. Complete ANOVA tables, including degrees of freedom, mean squares, F-values, and p-values, are provided in the Supplementary Material. Unless otherwise indicated, values for the factorial

design correspond to three independent experimental replicates per treatment ($n = 3$) (Table 1).

Coffee Fermentation

Fermentation was carried out by inoculating microencapsulated *M. caribbica* according to the experimental design (Sect. 2.10). Selected cherries were placed in 1 L plastic bioreactors and partially covered with distilled water to promote activation and release of the yeast. The bioreactors were incubated at 35 °C for 48 or 72 h, in triplicate. Fermentation temperature was set at 35 °C based on the thermal tolerance reported for fermentation-associated yeasts and preliminary trials performed with the starter used in this study. Criollo et al. [30] reported yeast adaptation across a broad temperature range up to 45 °C, supporting controlled fermentations above ambient conditions. Preliminary trials also indicated higher biomass production and improved adaptation of *M. caribbica* at 35–37 °C, which supported selection of 35 °C as the fermentation temperature. After fermentation, drying was conducted at 37 °C as a mild temperature selected to approximate practical drying ranges while limiting heat-driven changes that can negatively affect precursors and cup quality. Cherries were dried until reaching 11% moisture and a water activity of 0.55, which were used as endpoint criteria to standardize drying across treatments (Fig. 1).

Microbiological Parameters

Microbiological parameters were evaluated through colony counts, presumptive and confirmatory tests for coliforms, and growth kinetics of *M. caribbica*. For colony counts of lactic acid bacteria (LAB), Enterobacteriaceae, molds, and yeasts, serial dilutions up to 10^{-6} were prepared, and aliquots of 100 µL from the last three dilutions were inoculated on selective media: MRS with aniline blue (LAB), VRB (Enterobacteriaceae), PDA (molds), and malt extract agar (yeasts), following NTC 4519 (aerobic mesophilic counts), NTC 4516 (coliforms), and NTC 4132 (molds and yeasts). Plates were incubated at 37.5 °C for 48 h for bacteria and 60 h for molds and yeasts.

For presumptive and confirmatory tests for coliforms, aliquots of 1 mL from dilutions 10^{-1} to 10^{-3} were inoculated into lauryl sulfate broth with Durham tubes and incubated at 37.5 °C for 48 h; gas-positive tubes were further tested with Kovacs reagent for indole detection, where a red ring confirmed the presence of *E. coli* [32]. Growth kinetics of *M. caribbica* were determined by sampling at 0, 12, 24, 30, 36, 48, 60, and 72 h, followed by serial dilutions (10^{-3} to 10^{-5}) and spreading of 0.1 mL in triplicate onto malt agar plates. Plates were incubated at 37 °C for 72 h, and CFU counts were expressed as CFU/mL [31].

Physicochemical Parameters

Physicochemical parameters included measurements of moisture content, pH, reducing and total sugars, titratable acidity, and total soluble solids. Moisture content was determined using the AOAC 930.15 gravimetric method [33] with a thermobalance (OHAUS MB 23) at 105 °C for 16 h. The pH of homogenized coffee liquor was measured at 25 °C with a calibrated pH meter (Orion 710-A) in triplicate. Reducing sugars were quantified using the DNS method [34] with absorbance at 540 nm, while total sugars were determined by the anthrone method with absorbance at 620 nm; both analyses employed glucose standard curves ($R^2 = 0.999$). Titratable acidity was measured according to NTC 4623 [35] by titration with 0.1 M NaOH using phenolphthalein as an indicator, and results were expressed as milliequivalents of acetic acid per liter. Total soluble solids were determined with a digital refractometer (PCE Instruments, PCE-DR, Germany) and reported as °Brix.

Table 1 Experimental design for dry-processed coffee fermentation

Process Variables	Levels	Response Variables
Inoculum concentration (% w/w)	0, 3, 5, 8	Total soluble solids, Total and reducing sugars, pH, Titratable acidity, Microbiological and sensory analyses
Fermentation time (h)	48, 72	



Fig. 1 Experimental treatments subjected to fermentation

Scale-Up Test

The treatment selected for scaling corresponded to the condition that showed the most favorable physicochemical and microbiological performance in the laboratory-scale trials. This selection was based on an integrative decision matrix used as a decision-support tool to compare the response variables obtained for each treatment. The selected condition was then scaled up to ferment 20 kg of *C. arabica* var. Castillo cherries, previously selected and washed. Fermentation was carried out in a controlled system (CubaBIOTEC) consisting of a 70 L hermetic fermenter equipped with temperature, agitation, aeration, liquid feeding, and UV light control (Fig. 2).

After fermentation, drying, and hulling, green coffee was subjected to complementary analyses to evaluate the effect of *M. caribbica* encapsulated inoculum on ochratoxin A inhibition, phenolic content, antioxidant capacity, and sensory attributes. Total phenolic content was quantified using the Folin-Ciocalteu method with gallic acid as standard (20–300 µg), measuring absorbance at 760 nm and expressing results as gallic acid equivalents. Antioxidant capacity was determined using two methods: ABTS radical cation decolorization following standard methodology and the DPPH radical assay, with results expressed as Trolox equivalents in both cases. Ochratoxin A (OTA) was quantified by SynLab using high-performance liquid chromatography with fluorescence detection (HPLC-FLD), reported under method code HPLC-FL-207, within an ISO/IEC 17025:2017 accredited scope of service. The analytical report applies a reporting convention in which OTA concentrations below 5.0 µg/kg are classified as not detectable. This criterion was used to interpret OTA results. Sensory evaluation was performed by La Taza de Juan S.A.S. (Sevilla, Valle del Cauca), where coffee samples were roasted to a medium level, ground to medium–fine particle size, and evaluated by certified Q-grader Juan Sebastián Gómez Ocampo following SCA protocols (Specialty Coffee Association, s. f.). Attributes including fragrance/aroma, flavor, acidity, body, and balance were scored.

Results and Discussion

The results are presented in three sections. The first part describes the microbiological behavior of *Meyerozyma caribbica* and the initial characterization of coffee cherries, followed by the factorial experimental design used to evaluate the influence of fermentation time and inoculum concentration on physicochemical and microbiological parameters. The second part reports the statistical analysis of these variables, including growth dynamics and interaction effects. Finally, the third part addresses the optimization, validation, and performance of the selected inoculum during coffee fermentation, integrating complementary analyses of phenolic content, antioxidant capacity, and sensory quality.

Microcapsule Properties, Morphology, and Functional Performance

Microcapsules presented water activity of 0.57 ± 0.003. Moisture content ranged from 7.10 to 10.45% (wet basis), and the dried powder used for fermentation presented 1.3% moisture. Solubility reached 53.06 ± 0.54%, and wettability reached 31.46 ± 2.02 s, remaining below 120 s, supporting rapid dispersion in aqueous media. Microencapsulation percentage reached 86%, and viability in the encapsulated inoculum reached 8.20×10^8 CFU/g.

SEM micrographs showed irregular particles with a continuous coating. Surface views (Fig. 3a-c) did not show visible holes, and cross-sectional views (Fig. 3d–f) showed a compact coating layer. Fragmentation observed in Fig. 3a is associated with sample preparation. Particle size ranged from 5.25 to 10.6 µm, and microscopy at 100× after safranin



Fig. 2 Controlled fermentation system

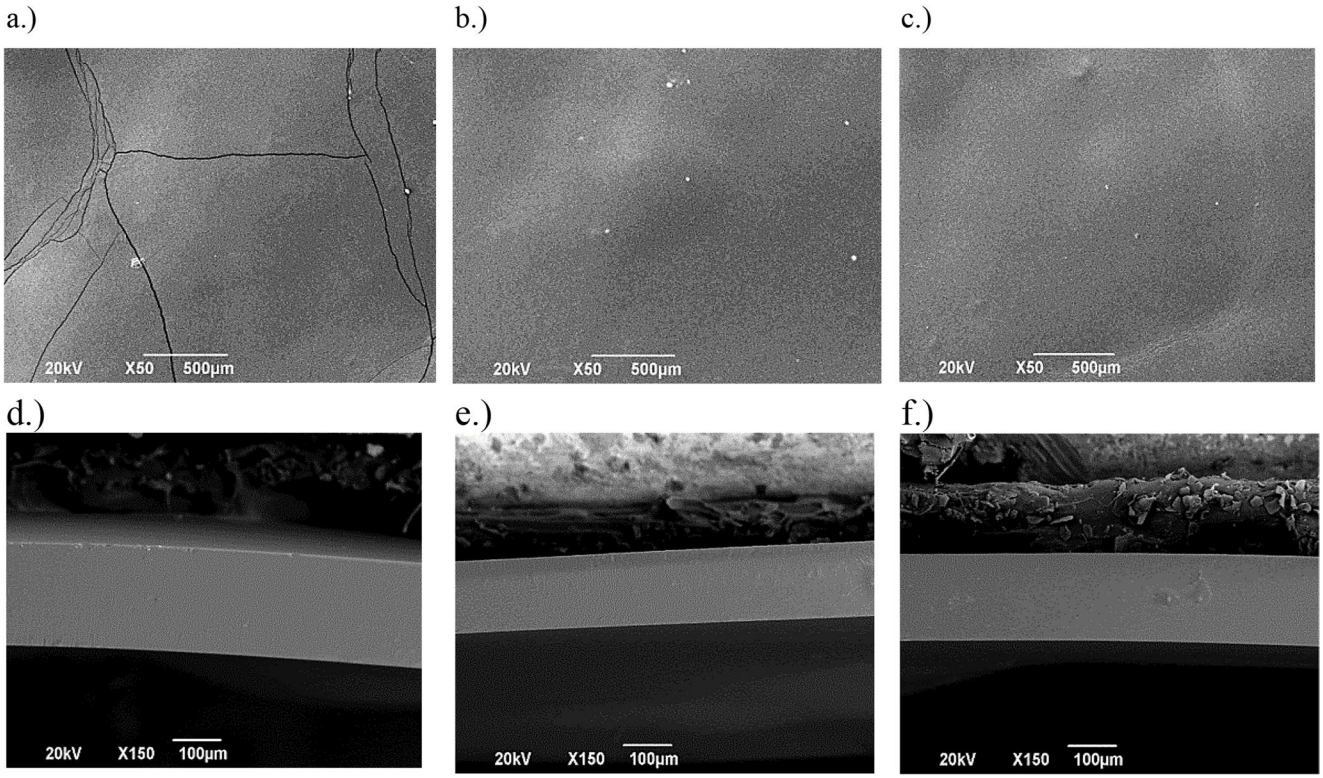


Fig. 3 Scanning electron microscopy (SEM) micrographs of microcapsules: surface views for (**a**–**c**); cross-sectional views for (**d-f**). Surface micrographs show a continuous coating without visible holes. Cross-sections show a compact coating layer; fragmentation in (**a**) is associated with sample preparation

staining showed diameters within agglomerates between 5.3 and 11 µm. The absence of surface holes supports the interpretation that EPS from *Weissella confusa* contributed to a sealing effect within the alginate–starch matrix, which can reduce surface discontinuities and support protection during drying and handling [28].

Microencapsulation percentage (86%) falls within the reference range reported for comparable systems [36]. Viability remained above 10^7 CFU/g, which matches commonly used benchmarks for starter preparations intended for fermentation [36] . Release testing in coffee matrix showed complete release between 5 and 9 min, reaching 7.6×10^8 CFU/g after release, supporting rapid initiation of fermentation after inoculation [30].

Water activity and moisture content contribute to microbiological stability during storage by limiting biochemical reactions, and these parameters have been discussed as relevant for dried starter preparations [28, 37]. A comparison with Criollo et al. [30] provides context for these values, since microencapsulated *Pichia kudriavzevii*, *Wickerhamomyces anomalus*, and *Lactobacillus plantarum* produced using the same formulation showed water activity between 0.42 and 0.46 and moisture around 7.6–7.8%. These results highlight the role of formulation and drying conditions in controlling water activity and moisture in encapsulated microbial starters.

Viability of the Microencapsulated Inoculum and Microbiological Analysis of Initial Coffee

Table 2 summarizes the viability of the *Meyerozyma caribbica* inoculum and the microbial counts of the initial coffee cherries.

The initial concentration and viability of the *M. caribbica* inoculum are critical for controlling the fermentation process, as the number of CFU directly influences microbial

Table 2 Viability of ***M. caribbica*** inoculum and microbial counts of initial coffee cherries

Parameter	Value (CFU/mL)
M. caribbica inoculum viability	3.8×10^8
Mesophilic bacteria	2.9×10^6
Enterobacteriaceae	2.0×10^6
Yeasts	3.0×10^6
Lactic acid bacteria (LAB)	2.0×10^6
Fungi	Not detected
Total coliforms (MPN)	>1100
Confirmatory test	*E. coli* detected

dynamics and fermentation efficiency [38, 39]. The viability obtained in this study was 3.8×10^8 CFU/mL, a value comparable to that described by Martins et al. [38]. In their study on yeast microencapsulation by spray drying for application in coffee-based fermentation media, they used inoculum with viabilities on the order of 10^7 CFU/mL. Their findings indicated that a high initial concentration not only enhances fermentation rate but also improves reproducibility and facilitates process scale-up. Siedler et al., (2020) further reported that high and sustained inoculum concentrations promote competitive exclusion and restrict the growth of undesirable microorganisms during fermentation.

According to De Bruyn et al. [40] and Elhalis et al. [1, 41], coffee cherries represent the main substrate for the growth of yeasts, bacteria, and filamentous fungi, with microbial composition determined by intrinsic characteristics of the bean, environmental conditions, and postharvest handling practices. In this study, the initial microbial load for mesophiles, Enterobacteriaceae, yeasts, and LAB was 2.9×10^6 CFU/g, 2.0×10^6 CFU/g, 3.0×10^6 CFU/g, and 2.0×10^6 CFU/g, respectively. These values are consistent with those reported by Sánchez-Riaño et al. [42], who evaluated the microbial load of coffee pulp after depulping and reported values of 1.1×10^6 CFU/g for mesophilic bacteria and 1.8×10^5 CFU/g for yeasts and molds. Similarly, Li et al., (2025) observed microbial analysis of coffee after harvesting and cherry selection, reporting yeast concentrations of 10^3 CFU/g.

The absence of fungi in the culture media observed in this study may be attributed to the application of good agricultural, hygienic, and handling practices during harvest. Studies such as Sánchez-Riaño et al. [42] demonstrated that sanitizing coffee cherries with potable water, combined with prior density selection, promotes stability of the fermentation process without significantly altering the initial microbiota. This procedure eliminates impurities, surface residues, and defective cherries, contributing to greater process control. Furthermore, Díaz et al. [43] reported that adequate hygiene and moisture control during coffee harvesting significantly reduce contamination by ochratoxin A, a compound directly associated with fungal growth and metabolic activity.

Regarding the presumptive test for coliforms, an MPN > 1100 was obtained, confirming the presence of total coliforms. These findings are consistent with those reported by Blandón Castaño et al. [44] in pulp samples, suggesting that the microbial load of coffee and its by-products follow a similar pattern. Previous studiesindicated that once harvested, coffee presents a considerable microbial load composed of microorganisms that drive the fermentation process [18, 40, 41]. Samples showing positive results with gas production were subjected to the indole confirmatory test with Kovacs reagent, suggesting the presence of *Escherichia coli* in the initial coffee. Subculture in EMB medium confirmed this result, with colonies showing metallic green sheen at dilution 10^{-1}, characteristic of bacteria with high efficiency in lactose fermentation such as *E. coli*, whose activity produces marked acidification of the medium [45]. Colonies with a wine-red color observed at dilution 10^{-2} likely correspond to other Enterobacteriaceae that ferment lactose more slowly [46].

Experimental Design and Preliminary Laboratory-Scale Trials

Table 3 summarizes the ANOVA p-values, with statistically significant effects ($p \leq 0.05$) shown in bold. The complete ANOVA outputs, including degrees of freedom, F-values, and p-values, are provided in the Supplementary Material. The factorial analysis confirmed that fermentation time and inoculum concentration had significant effects on both physicochemical and microbiological parameters. Fermentation time was the main factor influencing titratable acidity, pH, reducing sugars, and microbial populations, while inoculum concentration significantly affected sugar solubilization, and soluble solids. The interaction between the two factors was also significant for several variables, indicating that microbial activity and metabolite production depended on both fermentation duration and inoculation levels.

Fermentation time was the key driver of acidification (titratable acidity, pH) and sugar consumption (reducing and total sugars), consistent with studies showing that extended fermentations enhance metabolite release and microbial stabilization [47, 48]. Inoculum concentration significantly influenced sugar consumption and yeast proliferation, confirming its role in fermentation kinetics. The interaction of

Table 3 *p*-values from ANOVA showing the effects of fermentation time, inoculum concentration, and their interaction on the response variables (significant at $p \leq 0.05$)

Response variable	Model	Linear	Fermentation time (h)	Inoculum concentration (% w/w)	Interaction (Time × Inoculum)
Titratable acidity	**0.000**	**0.000**	**0.000**	0.064	**0.000**
Reducing sugars	**0.000**	**0.000**	**0.004**	**0.000**	0.130
Total sugars	**0.000**	**0.000**	**0.000**	**0.003**	**0.046**
pH	**0.000**	**0.000**	**0.000**	0.503	**0.000**
Soluble solids	**0.000**	**0.000**	1.000	**0.000**	**0.005**
Lactic acid bacteria	**0.016**	**0.007**	**0.001**	0.190	0.228
Mesophilic bacteria	**0.021**	**0.009**	**0.010**	**0.037**	0.277

time and inoculum was particularly relevant for pH, titratable acidity, and Enterobacteriaceae counts, reinforcing that fermentation outcomes are determined by their combined effect rather than a single factor.

These findings are consistent with reports indicating that controlled inoculation strategies shape microbial succession and metabolite synthesis, especially alcohols and esters during dry fermentations [25, 49]. These compounds contribute to desirable sensory profiles, including caramelized, red wine, and fruity notes, in contrast to profiles generated under shorter fermentations.

Preliminary trials were carried out to determine suitable fermentation times, evaluating four levels of the studied factor (Control, 3%, 5%, and 8%). During these trials, pH and soluble solids were monitored, providing critical insights into fermentation dynamics. The observed trends (Fig. 4) served as the basis for defining the experimental objective.

The evaluation of soluble solids (Fig. 4) showed that 24 h of fermentation was insufficient, as the limited decrease indicated restricted sugar utilization from pulp and mucilage, thereby limiting the formation of metabolites of interest [48]. In contrast, extending fermentation to 96 h produced a sharp decline in pH, surpassing the thresholds reported by Puerta [48] and compromising the sensory profile of the beverage.

Fermentation periods of 48 and 72 h promoted greater solubilization of simple sugars, supporting microbial metabolism and the synthesis of aromatic precursors [47]. Within this range, pH values remained in the optimal interval described by Puerta [50]. Comparable findings were reported by Palumbo et al. [49] and Vale et al. [25], who demonstrated that extended fermentations of 48 and 72 h enhanced the production of alcohols (1-hexanol, 2-heptanol, benzyl alcohol, phenylethyl alcohol, and 3-octanol) and esters (ethyl acetate and amyl acetate). These compounds contributed to sensory profiles characterized by caramelized, red wine, and fruity notes, contrasting with those obtained after shorter fermentations of 24 h.

Fermentation times of 48 and 72 h were established as the most suitable conditions for subsequent evaluations, as they balanced sugar utilization, microbial stability, metabolite synthesis, and the preservation of a desirable sensory profile.

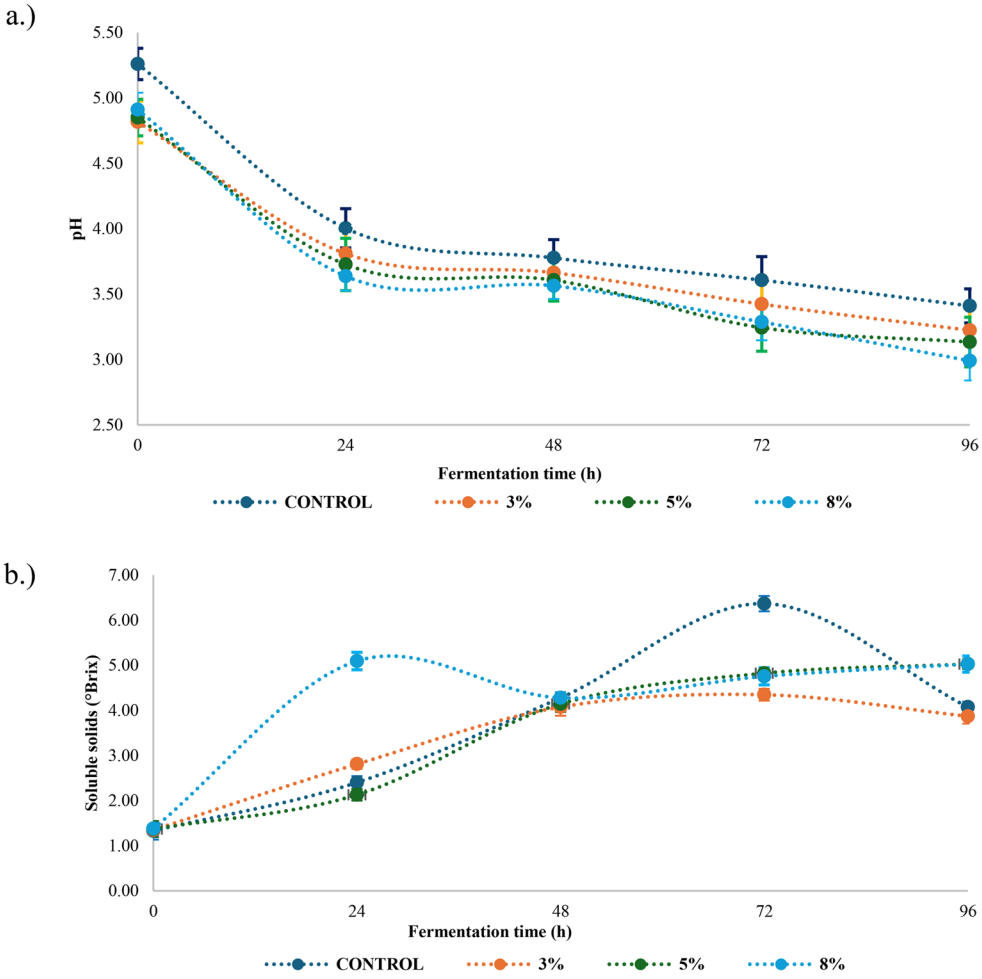


Fig. 4 Preliminary laboratory-scale fermentation trials of coffee cherries using different inoculum concentrations. Evolution of (**a**) pH and (**b**) soluble solids (°Brix) at different fermentation times and inoculum concentrations

Microbiological Parameters of Fermented Coffee

Table 4 presents the evolution of microbiological behavior of whole coffee cherries during fermentation, using different concentrations of the starter culture *Meyerozyma caribbica* (0%, 3%, 5%, and 8%) at two fermentation times (48 and 72 h). Average concentrations of yeasts, filamentous fungi, lactic acid bacteria, aerobic mesophiles, and Enterobacteriaceae are reported in colony-forming units per milliliter (CFU/g).

Figures S1–S13 (Supplementary Material) present the microbiological characterization and statistical analysis of fermented coffee under different inoculum concentrations and fermentation times.

Yeasts

Figure S1a shows the morphology of *Meyerozyma caribbica* under a stereoscope, allowing evaluation of its uniformity and macroscopic traits [51]. Colonies exhibited circular form, regular edges, convex surface, smooth and glossy texture, and creamy-white coloration, consistent with the description of Ebabhi et al. [52], who reported smooth, shiny, ovoid, or irregular colonies for the same yeast. Figure S1b shows yeast cells with oval to rounded structures, arranged individually or in small groups, indicative of active budding [52].

Figure S2 shows the average yeast colony-forming unit (CFU/g) counts during the fermentation of whole coffee cherries, comparing different inoculum concentrations (control, 3%, 5%, and 8%) at two fermentation times (48 and 72 h). Treatments showed significant differences, as presented in Table 3 and Figure S3a, both in main effects (fermentation time and inoculum concentration) and in their interaction, directly influencing yeast populations. Figure S3b shows a decline in yeast counts as fermentation progressed. Stanley et al. [53] attributed this reduction to substrate depletion, microbial competition, and accumulation of inhibitory metabolites such as ethanol, which disrupts cellular functions by integrating into membranes, increasing permeability, and altering enzymatic activity and ionic balance.

At 48 h, higher inoculum concentrations produced larger yeast populations, with peaks significantly higher than the control. This behavior was also influenced by fermentation system conditions. Antonio and Agüera [54], reported that fermentation type affects yeast viability; in closed systems, CO_2 accumulation induces physiological adaptations that allow certain strains to maintain viability, whereas in open systems, oxygen availability supports aerobic metabolism and population stability during extended fermentation. Figure S3a shows that the slope of the control and the 8% inoculum treatments reflect a sharper decline in yeast counts compared with other treatments, suggesting that the absence of inoculum or excessive concentrations reduce yeast stability during fermentation.

At 72 h, the control, 3%, and 8% inoculum treatments did not differ significantly, indicating that the 3% inoculum provided insufficient viable cells to sustain fermentation, while the high density in the 8% treatment likely caused rapid substrate depletion, limiting further growth. Westman et al. [55] showed that fermentations with high cell densities may experience reduced efficiency due to nutrient and oxygen limitations. In contrast, the yeast population in the 5% inoculum treatment remained more stable at both 48 and 72 h, reflecting a balance between substrate availability and microbial activity. This observation is consistent with Kaur et al. [56], who reported that high inoculum concentrations do not necessarily increase production and may reduce efficiency through nutrient competition and substrate depletion.

De Bruyn et al. [40] and Elhalis et al. [1, 41] emphasized the key role of yeasts in coffee fermentation, particularly in degrading mucilage and pulp pectin through extracellular enzymes such as polygalacturonase, pectin lyase, and pectin methylesterase. Pectin lyase breaks down pectin and releases unsaturated galacturonic acids; polygalacturonase hydrolyzes α-1,4 linkages of polygalacturonic acid; and pectin methylesterase catalyzes de-esterification of methoxyl groups, producing pectic acid and methanol. These processes, carried out by yeasts such as *M. caribbica* and *Pichia kluyveri* isolated from *C. arabica* and *C. canephora*, alter concentrations of soluble carbohydrates,

Table 4 Microbial dynamics during fermentation of coffee cherries with different concentrations of *M. caribbica* inoculum

Microorganisms	Time (h)	0% (Control)	3%	5%	8%
Yeasts	Initial	3.0×10^6	–	–	–
	48	3.8×10^8	8.5×10^8	9.9×10^8	2.2×10^9
	72	4.0×10^7	1.6×10^8	2.4×10^8	4.0×10^9
Fungi	48	Nd	Nd	Nd	Nd
	72	Nd	Nd	Nd	Nd
Mesophiles	Initial	2.9×10^6	–	–	–
	48	3.0×10^8	7.6×10^8	8.3×10^8	5.1×10^8
	72	3.9×10^7	1.6×10^8	5.1×10^8	4.7×10^8
Lactic acid bacteria	Initial	2.0×10^6	–	–	–
	48	1.2×10^9	7.1×10^8	8.1×10^8	2.8×10^8
	72	1.6×10^7	1.2×10^8	2.2×10^8	1.4×10^8
Enterobacteriaceae	Initial	2.0×10^6	–	–	–
	48	4.0×10^4	Nd	Nd	Nd
	72	Nd	Nd	Nd	Nd
Total coliforms	Initial	>1100	–	–	–
	48	192	<3	<3	<3
	72	<3	<3	<3	<3

Results expressed as mean concentrations (CFU/g), except for coliforms (MPN/mL). Nd: not detected

organic acids, and volatile compounds in pulp, favoring the formation of aromatic precursors [57].

Lactic Acid Bacteria (LAB)

Shen et al. [22] indicated that lactic acid bacteria represent a significant proportion of the coffee fruit microbiota, since their metabolic activity plays a central role in mucilage degradation, volatile compound generation, and pH modification. Figure S4 shows LAB growth on MRS medium with aniline blue, where colonies with blue halos indicate lactic acid production [58]. Figure S5 presents LAB CFU/g counts at 48 and 72 h, and Figure S6 shows factorial interaction and main effects. Fermentation time was significant ($p \leq 0.05$), with counts decreasing as fermentation advanced. The pronounced reduction in the control at 72 h correlated with lower pH values.

These results align with Gutiérrez-Sarmiento et al. [59], who reported optimal LAB growth at near-neutral pH with moderate oxygen incorporation. Stability in inoculated treatments is explained by yeast–LAB cooperation: yeasts release amino acids (glutamine, glutamate, proline, phenylalanine) that serve as nutrients for LAB, while LAB acidify the medium, supporting yeast metabolism and mucilage degradation [60, 61]. LAB also contribute to biocontrol by producing organic acids that inhibit fungal growth [64].

Mesophilic Microorganisms

Figure S7 shows growth of mesophiles on PCA medium, while Figure S8 presents CFU/g counts at 48 and 72 h, and Figure S9 shows factorial interactions and main effects. At 48 h, inoculated treatments had higher counts than the control. Statistical analyses revealed that both fermentation time and inoculum concentration had significant effects ($p \leq 0.05$). However, the absence of interaction suggests that these effects are independent, meaning that certain combinations may yield similar outcomes due to balancing individual effects.

At 48 h, mesophilic concentrations were higher in all inoculated treatments compared with the control. By 72 h, a generalized decline was observed. Puerta et al. [62] associated this reduction with the progressive acidification caused by organic acid production during fermentation, creating unfavorable conditions for mesophiles. Microbial competition and nutrient availability also contributed to this decrease.

Peñuela-Martínez et al. [63] reported that mesophilic populations can remain stable during fermentation under controlled conditions at 30 °C, attributed to thermotolerance, which explains the high concentrations observed. Moreover, yeast inoculation appeared to favor mesophilic growth, possibly due to metabolites that stimulate development. This interaction may reflect metabolic synergy between yeasts and mesophiles, including LAB as an important component. Pereira et al. [61] highlighted that yeasts release amino acids serving as essential nutrients for LAB growth and activity.

Fungi

Fungi are relevant in many biotechnological processes due to their ability to synthesize enzymes and secondary metabolites [64]. In coffee fermentation, their development is influenced by environmental and biological conditions of the fermentation ecosystem, including natural vectors such as the coffee berry borer (*Hypothenemus hampei*) [22, 24]. Species such as *Penicillium*, *Fusarium*, *Cladosporium*, *Aspergillus*, *Pestalotia*, and *Paecilomyces* can contribute to mucilage degradation. However, Shen et al. [22] reported that in dry fermentation, fungal populations tend to increase progressively from harvest to drying, raising the risk of contamination with mycotoxins. This contamination is mainly due to filamentous fungi, with *Aspergillus*, *Penicillium*, *Cladosporium*, and *Fusarium* as the primary agents [24]. Among these mycotoxins, ochratoxin A stands out for its high toxicity, being classified as teratogenic, carcinogenic, hepatotoxic, neurotoxic, and nephrotoxic [65, 66].

No fungal growth was detected in any treatments of this study, which could be attributed to either the initial absence of fungi or microbial competition in the fermentative environment that inhibited their development [67]. Previous research indicated that yeasts and LAB can restrict fungal growth through the production of antifungal metabolites such as 2-phenylethanol, isoamyl alcohol, 4-hydroxyphenylethanol, isoamyl acetate, and ethyl acetate, which inhibit both mycelial growth and mycotoxin synthesis [68].

Some yeasts act as biological control agents through the production of lytic enzymes such as proteases, chitinases, and glucanases, which degrade structural components of other microorganisms, thereby limiting their development [24]. *M. caribbica* has demonstrated strong antagonistic capacity, showing notable biocontrol effects in fruits such as kiwi, mango, jujube, and passion fruit [68]. According to Salazar-Hernández et al. [69], M. *caribbica* can secrete hydrolytic enzymes including chitinase, β-1,3-glucanase, and N-acetyl-β-D-glucosaminidase, which inhibit fungal hyphae growth.

Enterobacteria

Enterobacteria comprise a group of pathogenic microorganisms that pose a significant risk to human health [45, 70]. Braga et al. [71] reported their natural presence in farms and

coffee plants. In their study, the Enterobacteriaceae family—including *Enterobacter*, *Citrobacter*, *Klebsiella*, and *Pantoea*—was predominant during fermentation. Figure S10 shows enterobacteria on MacConkey medium, and Figure S10 presents their dynamics during fermentation. At 48 h, the control treatment showed 4.00×10^4 CFU/g of enterobacteria, although no growth was observed in inoculated treatments with *M. caribbica*. This suggests that yeasts may inhibit bacteria through antimicrobial metabolite production or nutrient competition. Olstorpe et al. [72] demonstrated that yeasts such as *Hansenula anomala* (also known as *Pichia anomala*) inhibited enterobacteria including *E. coli*, *Klebsiella pneumoniae*, *Enterobacter sakazakii*, and *Serratia marcescens*, mainly through volatile antimicrobial compounds such as ethyl acetate, which suppress both molds and bacteria. Braga et al. [71] also reported that enterobacteria such as *Klebsiella* and *Enterobacter* can participate in coffee fermentation via the Embden-Meyerhof pathway, releasing CO_2, H_2, acetone, and 2,3-butanediol, compounds that negatively affect beverage sensory quality.

Although species such as *Enterobacter*, *Klebsiella*, and *Pantoea* can produce enzymes including pectinases, xylanases, and pectin methylesterases that degrade complex carbohydrates and release simple sugars [73], in treatments where these bacteria predominated, no significant increase in reducing sugars was observed, suggesting low enzymatic activity compared with the starter inoculum.

Coliforms, members of Enterobacteriaceae, are widely used as indicators of fecal contamination, since their detection reflects deficiencies in hygiene practices along the production chain, compromising both quality and safety of the final product [32, 45].

At 48 h, the control treatment showed 192 MPN/mL of total coliforms, whereas no coliforms were detected in inoculated treatments, suggesting a possible inhibitory effect of the starter culture. Previous studies have reported noted that yeasts of the genus *Saccharomyces* produce antimicrobial compounds such as organic acids that inhibit coliforms by acidifying the medium. The absence of coliforms in all treatments at 72 h agrees with Puerta [62], who stated that decreasing pH creates a hostile environment for coliforms while favoring acidophilic microorganisms such as yeasts and LAB.

The negative indole test in the control treatment at 48 h (Figure S12) suggests the absence of *E. coli*, which is typically identified by its ability to produce indole from tryptophan (Baldeón Beltrán, 2013). This absence may be attributed to inhibition of *E. coli* by acidic conditions generated during fermentation or by competition for nutrients. Nonetheless, gas production indicates the presence of lactose-fermenting coliforms, since non-fecal enterobacteria such as *K. pneumoniae*, *K. oxytoca*, and *E. cloacae* can ferment lactose and produce gas without indole [70].

Growth Kinetics

The established concentrations of the starter culture directly influenced the progression of fermentation, determining both microbial load and persistence of microorganisms over time. This process was further modulated by several factors, including the production of metabolites and antimicrobial peptides, as well as competition for available substrates in the coffee matrix [74, 75].

Figure 5 shows the evolution of *Meyerozyma caribbica* concentrations (CFU/mL) during fermentation of whole cherries at three inoculum levels (3%, 5%, and 8%). This graphical representation illustrates yeast dynamics as a function of time and initial inoculum concentration.

In Fig. 5, yeast populations inoculated at the three different concentrations exhibited an exponential growth phase until reaching a maximum peak, followed by a stationary phase and subsequent decline. The 8% inoculum treatment

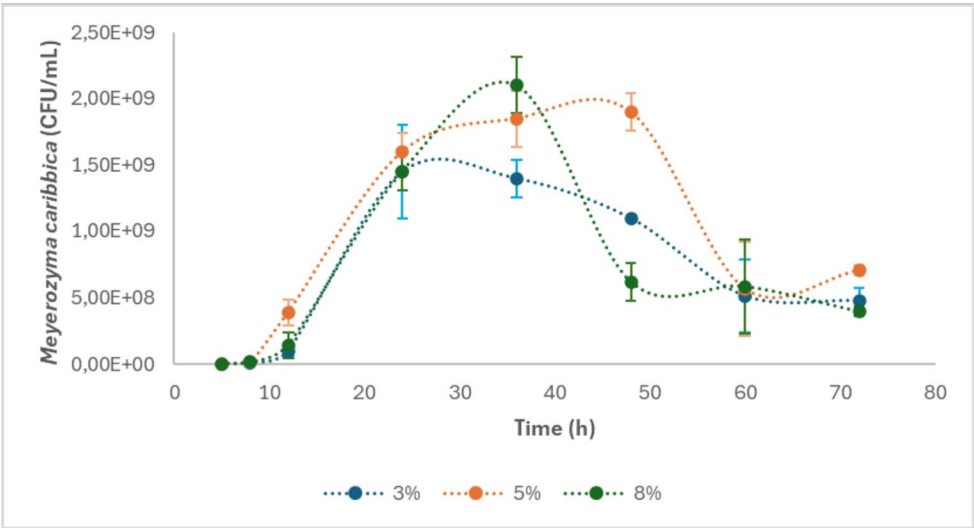


Fig. 5 Growth kinetics of microencapsulated *Meyerozyma caribbica*

reached the highest yeast concentration at 36 h (2.10×10^9 CFU/g). Subsequently, a substantial decrease in cell density was observed, suggesting either depletion of available sugars or inhibition caused by accumulated metabolites [53].

In the 5% inoculum treatment, microbial populations entered exponential growth up to 24 h, followed by a stationary phase that peaked at 48 h (1.90×10^9 CFU/g), after which a gradual decline occurred, reflecting greater stability of the fermentative system. This behavior agrees with Lessa da Silva et al. [57], who also reported maximum populations at 48 h in coffee fermentations, a critical stage for depolymerization of pectin in mucilage and pulp through enzymes such as pectinases and cellulases.

At 24 h, the 3% inoculum treatment reached its maximum population (1.5×10^9 CFU/g). Up to this point, its microbial growth rate was comparable to the 8% inoculum treatment. However, the yeast population then declined progressively, likely due to a lower initial biomass that limited both colonization rate and metabolite production [76].

Physicochemical Parameters of Fermented Coffee

Moisture

Dry matter determinations showed moisture contents on a wet basis of 68.39% ± 0.0133 and 67.23% ± 0.0105 for the coffee bean and cherry, respectively. These values fall within the range reported by Pereira et al. [77] for harvested coffee fruits and are consistent with Coelho et al. [78], who indicated that cherry moisture at harvest varies between 30% and 65% depending on ripeness. The slightly lower moisture content observed in whole cherries compared with beans is explained by the inclusion of additional tissues, mainly pulp and mucilage, which contain higher proportions of dry matter (approximately 29% and 5% on a dry weight basis, respectively). Their presence increases the total solid fraction of the sample, leading to a relative reduction in moisture content on a wet basis [41].

pH and Titratable Acidity

Measurement of pH is a reliable parameter for achieving greater consistency and control during fermentation, preventing both under- and over-fermentation, conditions that directly affect final coffee quality [79]. From the analysis presented in Table 3, the interaction between fermentation time and inoculum concentration was statistically significant ($p \leq 0.05$), indicating that the effect of inoculum concentration on pH depended on fermentation time, and conversely, the effect of fermentation time depended on inoculum concentration.

This significant interaction explains the patterns shown in Figure S13a, where pH trends varied notably between 48 and 72 h at different inoculum levels. Fermentation time was identified as a statistically significant factor (Figure S13b), confirming that process duration consistently affected pH regardless of inoculum concentration. This trend is further shown in Figure S14, where pH values at 72 h were lower than those at 48 h across all treatments. In contrast, inoculum concentration alone had no significant effect ($p > 0.05$), suggesting that variations in inoculum level do not independently produce significant changes in pH. The observed differences in pH at different concentrations were primarily mediated by the interaction with fermentation time.

As shown in Figure S14, the absence of inoculum at 72 h resulted in the lowest pH (3.30), comparable with values reported by Galarza and Figueroa [80] for spontaneous fermentations, where a pH of 3.5 was observed at the same stage. According to these authors, pH reduction is mainly attributed to the activity of endophytic mesophiles present in the cherry, which degrade pulp and mucilage substrates, including cellulose, highly methyl-esterified pectic substances (galacturonic acids), and sugars through extracellular enzymatic activity, leading to mucilage acidification [41, 57].

In the control treatment, acidification was markedly higher compared with inoculated treatments. This effect could be attributed to the absence of the *M. caribbica* starter, which allowed the native acidifying microflora to predominate, increasing acid release and accelerating pH decline [1]. These results are consistent with Elhalis et al. [1], who demonstrated that inhibition of yeast growth using antifungals in wet fermentation increased organic acid concentrations, particularly acetic and butyric acids. By contrast, the addition of moderate inoculum concentrations (3–5%) slowed pH reduction, suggesting mechanisms of competitive exclusion and physiological adaptation of inoculated yeasts [10, 81]. According to Elhalis et al. [41], yeasts compete effectively for nutrients and limit pectinolytic activity of bacteria, reducing production of acidifying metabolites by the autochthonous microbiota, composed mainly of lactic acid bacteria (*Lactobacillus*, *Lactococcus*, *Leuconostoc*) and acetic acid bacteria (*Gluconobacter*, *Acetobacter*) [40, 82, 83]. This competitive advantage is reinforced by the high acid tolerance of yeasts, which allows them to remain active under low pH conditions while inhibiting acid-sensitive bacteria [79].

However, at 8% inoculum and 72 h of fermentation, pH decreased again, suggesting that high microbial density leads to greater pectinolytic activity. This interpretation is consistent with Lessa da Silva et al. [57], who demonstrated that maximum yeast populations in dry coffee fermentation are decisive for depolymerization and hydrolysis of pectin

in mucilage and pulp. This process is associated with the synergistic action of enzymes such as polygalacturonase, pectin lyase, pectin methylesterase, and cellulases.

Preference of microorganisms for simple sugars over polysaccharides favored the production of organic acids, mainly lactic and acetic acids, which led to increased titratable acidity as fermentation time extended (Figure S15 and S16). This behavior revealed the expected inverse correlation with pH, illustrated in Figure S14 [41, 80]. According to Nakyinsige et al. [83] and Martins et al. [39], the rise in titratable acidity results from microbial degradation of pectin into simple sugars, subsequently metabolized to organic acids. Bressani et al. [81] reported detection of multiple organic acids during yeast-inoculated dry fermentation, including acetic, citric, malic, succinic, lactic, isobutyric, and propionic acids, with acetic acid being the most abundant.

Reducing and Total Sugars

Pereira et al. [77] emphasized that aroma development in coffee depends on the presence of chemical precursors in the bean, particularly insoluble carbohydrates such as cellulose and hemicellulose, and soluble sugars including arabinose, fructose, galactose, glucose, sucrose, raffinose, and stachyose [84]. Low-molecular-weight sugars such as sucrose, glucose, and fructose are critical during roasting, as they are precursors of acids and volatiles in Maillard, Strecker, and pyrolytic reactions [1]. Polysaccharides in the bean are also essential for volatile retention, directly influencing flavor perception [77].

Statistical analysis of reducing sugars showed that the interaction between fermentation time and inoculum concentration was not significant ($p > 0.05$). However, both factors independently showed significant effects ($p \leq 0.05$), as seen in Figure S17, confirming that each factor alone determined by reducing sugar concentrations.

The highly significant effect of inoculum concentration explains the clear increase in reducing sugar values with higher inoculum levels (Figures S17 and S18). This behavior suggests that higher yeast inoculum enhances hydrolysis of complex polysaccharides such as pectin, cellulose, and sucrose through enzymes like invertase, releasing monomers such as fructose, glucose, and rhamnose [15]. These findings are consistent with Elhalis et al. [1], who reported that yeast inoculation facilitates degradation of complex carbohydrates into monosaccharides through metabolic activity.

At the same time, fermentation time significantly affected reducing sugar concentrations, showing a gradual decline as fermentation progressed (Figure S18). The sharp decrease at 72 h, particularly in the control and the 8% treatment, suggests progressive sugar consumption by microorganisms as the main carbon source during prolonged fermentation [1]. Pereira et al. [77] noted that yeasts produce multiple low-molecular-weight compounds, including esters, higher alcohols, aldehydes, ketones, and terpenoids, during mucilage removal as a result of sugar metabolism.

In ripe *C. arabica* fruits, cell wall polysaccharides (CWPs), mainly galactomannans and arabinogalactans, represent 48–60% of dry matter and serve as key reserves [85]. The significant decrease in total sugars between 48 and 72 h (Figures S19 and S20) reflects microbial metabolism and progressive degradation of CWPs and sucrose.

During the first 48 h, microorganisms favored hydrolysis of complex compounds, releasing simple sugars and increasing total sugar concentration, particularly at 8% inoculum. Prakash et al. [15] argued that sugars are the first substrates metabolized during coffee fermentation, with yeasts playing the primary role through hydrolytic enzymes such as amylases, pectinases, and cellulases, with pulp as the main substrate. At 72 h, the high reduction in total sugar concentrations indicated a metabolic shift toward consumption for cell growth and volatile metabolite synthesis associated with aroma and sensory profile [86]. This transition is consistent with microbial adaptive responses under nutrient stress, intensifying degradation of alternative carbon sources. Polanía et al. [87, 88] also observed that sugars were consumed for microbial growth while significant secondary metabolites, such as ethanol and acetic and lactic acids, were produced. Bruyn et al. [40] further noted that under prolonged fermentation, anoxia caused by immersion can trigger endosperm germination and anaerobic metabolism, consuming carbohydrates through glycolysis.

The high total sugar concentrations observed at 8% inoculum may be attributed to enhanced hydrolytic activity during early stages, while lower values at 3% may reflect imbalances between enzyme production and substrate availability. Mardhatilah et al. [89] reported that limited yeast concentrations result in insufficient carbohydrate degradation. Interestingly, the control treatment at 48 h revealed native microflora with hydrolytic activity comparable to that of the 5% inoculum, whereas sharper sugar reductions at 8% suggested that sugar consumption rate was proportional to population density [89].

Total Soluble Solids (TSS)

Quantification of TSS is a relevant criterion for determining the endpoint of fermentation, since their concentration reflects not only sugars but also organic acids, vitamins, amino acids, and phenolic pectins, whose variation is

closely related to mucilage degradation and fermentation progression [15, 90].

Statistical analysis of TSS revealed that both inoculum concentration ($p \leq 0.05$) and its interaction with fermentation time ($p \leq 0.05$) were significant. In contrast, fermentation time as an independent factor had no significant effect ($p > 0.05$) (Figure S21b). The interaction explained the nonlinear patterns shown in Figure S21a. At 48 h, the 8% inoculum treatment showed the highest TSS values (5.8–6.0 °Brix), whereas at 72 h, the control treatment registered the highest values (5.3 °Brix) (Figure S22).

The differential behavior according to inoculum concentration revealed specific metabolic dynamics mediated by microbial diversity and population density [83]. The 8% inoculum treatment showed intense enzymatic activity during the first 48 h, favoring rapid solubilization of sugars, followed by metabolic conversion to alcohols that caused a significant reduction in TSS at 72 h [91]. In contrast, the control, 5%, and 3% treatments showed increases in TSS between 48 and 72 h, with progressively lower release rates. Nakyinsige et al. [83] attributed such variations to microbial degradation of sugars into organic acids. Polanía et al. [19, 20] suggested that TSS stability may result from the selective sugar preferences of LAB, which readily utilize glucose but have limited capacity to metabolize sucrose and fructose.

Finally, factors such as pulp and mucilage thickness and variations in bean size and density across treatments influenced fluctuations in TSS during fermentation [63].

Scaling Test

The physicochemical and microbiological results obtained in the factorial design were integrated through a decision matrix (Table 5) to support selection of the treatment used for scaling. This matrix was used as a decision-support tool for comparing the overall performance of the treatments and did not constitute a statistical optimization method. Based on this integrative comparison, the optimal condition for the scale-up stage was selected, and the subsequent complementary analyses focused on phenolic compounds, ochratoxin A, antioxidant capacity, and sensory evaluation.

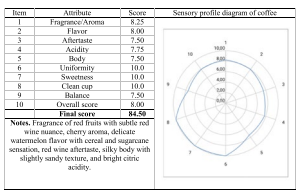
From the results presented in Table 6, the treatment with 5% *M. caribbica* at 48 h emerged as the most suitable for scaling, achieving the highest consolidated score (15 points). This treatment showed superior performance in key variables such as yeast viability and stability during fermentation, antagonistic activity against Enterobacteriaceae, formation of a balanced consortium with lactic acid bacteria, maintenance of pH at optimal levels, and efficient degradation and utilization of sugars for

Table 5 Decision matrix

Decision criteria	Treatments							
	48 h				72 h			
	Control	3%	5%	8%	Control	3%	5%	8%
Viability and stability of *M. caribbica* during fermentation	N/A	2	3	1	N/A	2	3	1
Antagonistic activity of *M. caribbica* against Enterobacteriaceae	N/A	3	3	3	N/A	3	3	3
Population consortium between *M. caribbica* and LAB	N/A	2	3	1	N/A	1	3	2
Optimal pH (pH: 3.5)	2	3	3	3	1	2	2	1
Degradation of complex sugars (TSS)	2	1	1	3	2	1	1	3
Utilization rate of complex and simple sugars by *M. caribbica* for organic acid production (pH, titratable acidity)	N/A	1	2	3	N/A	2	3	3
Consolidated score	4	12	15	14	3	11	15	13

Note: The scoring system was structured hierarchically into three levels: 3 (High), 2 (Medium), and 1 (Low).

Table 6 Sensory evaluation results by *La Taza de Juan S.A.S*



Item	Attribute	Score
1	Fragrance/Aroma	8.25
2	Flavor	8.00
3	Aftertaste	7.50
4	Acidity	7.75
5	Body	7.50
6	Uniformity	10.0
7	Sweetness	10.0
8	Clean cup	10.0
9	Balance	7.50
10	Overall score	8.00
	Final score	**84.50**

Sensory profile diagram of coffee

Notes. Fragrance of red fruits with subtle red wine nuance, cherry aroma, delicate watermelon flavor with cereal and sugarcane sensation, red wine aftertaste, silky body with slightly sandy texture, and bright citric acidity.

organic acid production. Moreover, the 48 h treatment offers additional advantages by reducing fermentation time, which optimizes the process from both technical and operational perspectives without compromising final product quality.

Sensory Analysis

The sensory analysis of the scaled treatment classified the product as specialty coffee (see Table 6), according to the standards of the Specialty Coffee Association (SCA). The evaluation was carried out by a certified Q Grader at the laboratory *La Taza de Juan S.A.S.*, obtaining a final score of 84.50 points.

Uniformity, clean cup, and sweetness reached the maximum score of 10.0, reflecting controlled fermentation conditions, proper cherry selection at optimal ripeness, good laboratory practices, and the purity of the starter culture [87]. The fragrance/aroma parameter scored 8.25, highlighting fruity notes such as cherry, red fruits, and red wine. Bressani et al. [92] reported that *M. caribbica* enhances the generation of aromas associated with red fruits and caramelized sugars. Similarly, Pereira et al. [77] indicated that *M. caribbica* strains contribute to volatile synthesis, including organic acids, higher alcohols (phenylethanol), and esters (benzyl benzoate, ethyl hexadecanoate), all linked to fruity and floral sensory profiles. Liu et al. [93] demonstrated the role of yeasts in improving sensory profiles; *Saccharomyces cerevisiae*, used as starter, produced volatiles such as 3-methylbutanol and 2-phenylethanol, precursors of isoamyl acetate and 2-phenylethyl acetate, which impart ripe fruit, cherry, and floral notes.

The flavor parameter scored 8.0, with descriptors of watermelon and complementary cereal-sugarcane notes. Tieghi et al. [94] emphasized that flavor arises from the integration of gustatory sensations and retronasal aromas. These cereal descriptors may be linked to pyrroles, volatile compounds mainly generated during roasting [95, 96].

Acidity scored 7.75, described as bright and citric. Echeverri-Giraldo et al. [97], working with *C. arabica* var. Castillo, identified lactic acid, phosphoric acid, and 3,5-dicaffeoylquinic acid (3,5-di-CQA) as key compounds correlated with acidity perception, directly contributing to this sensory attribute. Aftertaste scored 7.50, reflecting persistence of positive taste and aroma after consumption [94], with subtle red wine notes possibly linked to chlorogenic acid-derived lactones such as 3,4-O-caffeoyl-γ-quinide, which influence flavor stability [88, 98]. Body was rated 7.50, described as silky with a slightly sandy texture. This attribute may result from multiple factors. Bressani et al. [92] showed that fermentation with *M. caribbica* produced a dense and structured body. Díaz et al. [43] noted that thermal decomposition of phenols during roasting releases caffeine, which contributes to intensity and body. [65, 79]. The combined effects of varietal biochemistry, fermentation, and roasting likely explain the silky yet sandy texture.

Balance was rated 7.50, reflecting a harmonious interaction of attributes and an overall positive perception [94].

Comparatively, Polanía et al. [19, 20] reported 81.75 points for spontaneous fermentation of Castillo coffee. The differences between their results and the present study were most evident in fragrance/aroma, flavor, acidity, and balance, as well as in specific notes of red fruits, red wine, cherry, watermelon, silky body, and bright citric acidity. These variations underscore the influence of fermentation and processing conditions on the organoleptic profile of coffee.

Total Phenols and Antioxidant Capacity

Results of total phenolic content and antioxidant capacity, measured by ABTS and DPPH assays, are summarized in Table 7 as mean ± SD ($n = 3$). Total phenolic content reached 2228.6 ± 215.4 mg GAE/100 g, while antioxidant capacity reached 4072.5 ± 481.9 mg Trolox/100 g by ABTS and 1939.2 ± 218.4 mg Trolox/100 g by DPPH. These values fall within the ranges reported for coffee matrices in the literature [99–101].

Values are reported as mean ± SD of three independent measurements ($n = 3$).

The measurements correspond to green coffee and therefore reflect the combined effect of dry processing and controlled fermentation, but they do not capture the compositional shifts that occur during roasting. Roasting can decrease the concentration of native phenolics, including chlorogenic-related structures, through thermal degradation and transformations associated with Maillard reactions and melanoidin formation [102, 103]. Roast degree further modulates retention, and light roasts often show higher polyphenol retention because of lower thermal exposure combined with concentration effects linked to moisture loss [104]. Processing method also contributes to variability in phenolic content, since conditions that change contact time with water and fermentation intensity can influence extraction and degradation patterns [77, 102]. This context supports interpretation of the values reported here as a baseline

Table 7 Total phenolic content and antioxidant capacity of green coffee measured by ABTS and DPPH assays

Sample	Total phenols (mg GAE/100 g)	Antioxidant capacity ABTS (mg Trolox/100 g)	Antioxidant capacity DPPH (mg Trolox/100 g)
Green coffee	**2228.6 ± 215.4**	**4072.5 ± 481.9**	**1939.2 ± 218.4**

after processing and fermentation rather than a proxy for the roasted coffee composition.

Differences between ABTS and DPPH results are expected because each assay responds to different antioxidant fractions. ABTS captures both hydrophilic and lipophilic antioxidant capacity, while DPPH shows higher sensitivity toward lipophilic components, which can yield lower absolute values [103]. The ABTS and DPPH results reported here therefore remain method-consistent and comparable to values reported for fermented coffees inoculated with lactic acid bacteria and yeast consortia (Therdtatha et al., 2023).

Ochratoxin A

One of the main constraints in international coffee trade is contamination with ochratoxin A (OTA) [66]. It has been estimated that about 10% of coffee batches present detectable levels of this mycotoxin [105]. The presence of OTA not only compromises quality and safety but also reduces sensory acceptance in specialty markets [66]. Its monitoring and control are therefore essential for food safety under increasingly strict international regulations. In Table 8, the results of the OTA determination for the scaled treatment are shown.

The results indicated minimal detection of mycotoxin-related compounds in the processed samples. OTA was reported as not detectable, based on the reporting criterion of the accredited assay, which classifies values below 5.0 µg/kg as not detectable. This interpretation was considered in relation to European Union maximum levels for OTA in roasted and ground coffee, as established in Commission Regulation (EU) 223/915 [106]. The regulatory limit for this product category falls within the range of 3.0–5.0 µg/kg; therefore, the processed samples remained below the maximum levels permitted under EU criteria.

Absence of OTA also suggests adequate postharvest management that prevented favorable conditions for filamentous fungi such as *Aspergillus* and *Penicillium*. This is critical since OTA is thermostable and not fully degraded even at 250 °C [65, 107], meaning its presence in green coffee irreversibly compromises final quality. Additional factors such as harvest season, storage time, and storage conditions significantly influence OTA accumulation [65]. Effective control therefore requires not only controlled fermentation but also good practices during drying and storage to ensure optimal water activity and inhibit fungal growth, thereby minimizing the risk of OTA contamination [108].

Table 8 Determination of OTA in the scaled treatment

Analysis	Unit	Specification	Result	Method
OTA	N/A	Maximum 5.0 µg/kg	Not detected	HPLC-FL-207

Conclusions

The application of starter cultures in coffee fermentation was proposed as a strategy to reduce processing time, improve control, and optimize the sensory quality of the final product. In this context, the use of microencapsulated *Meyerozyma caribbica*, isolated from the fruit itself and applied at 5% for 48 h, enabled successful completion of fermentation without inducing over-fermentation, as evidenced by physicochemical parameters, particularly pH. Microencapsulation also contributed to extending the shelf life of the culture for up to six months, facilitating its handling and application under controlled conditions, while reducing the risk of contamination. This microbiological control had a positive impact on key sensory attributes, including sweetness, clean cup, uniformity, aroma, flavor, body, aftertaste, balance, and overall score. Additionally, the use of pulp as substrate in dry processing supported the reduction of organic waste, promoting full utilization of the fruit. Therefore, this strategy represents a promising alternative to transform a traditionally variable process into a technologically controlled approach focused on improving cup quality and process sustainability. Future research should further explore the characterization of microbial isolates and define optimal industrial-scale conditions to shorten fermentation times using selected starter cultures.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s11483-026-10133-9.

Author Contributions V.E.R. conducted the experimental work, collected data, contributed to statistical analysis, prepared figures, and participated in writing the manuscript. J.D.T.M. conducted the experimental work, collected data, contributed to statistical analysis, prepared figures, and participated in writing the manuscript. C.R.T. designed the study, supervised the experimental process, and participated in writing the manuscript. G.B. contributed to microbiological analyses, interpretation of results, and writing of the manuscript. J.L.P.D. coordinated the project, provided resources, performed statistical analysis together with V.E.R. and J.D.T.M., critically revised the manuscript, and contributed to writing. All authors contributed to writing and reviewing the manuscript, and approved the final version.

Funding Open Access funding provided by Colombia Consortium. The research leading to these results received funding from Universidad del Valle under the Internal Call 140-2022 (Project CI 21215), led by José Luis Plaza Dorado.

Data Availability All data supporting the findings of this study are available within the paper and its Supplementary Information.

Declarations

Competing interests The authors declare no competing interests.

Open Access This article is licensed under a Creative Commons Attribution 4.0 International License, which permits use, sharing, adaptation, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons licence, and indicate if changes were made. The images or other third party material in this article are included in the article's Creative Commons licence, unless indicated otherwise in a credit line to the material. If material is not included in the article's Creative Commons licence and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder. To view a copy of this licence, visit http://creativecommons.org/licenses/by/4.0/.

References

1. H. Elhalis, J. Cox, D. Frank, J. Zhao, The crucial role of yeasts in the wet fermentation of coffee beans and quality. Int. J. Food Microbiol. **333**, 108796 (2020). https://doi.org/10.1016/j.ijfoodmicro.2020.108796
2. S. Orecchio, D. Amorello, S. Barreca, Analysis of contaminants in beverages. En A. M. Grumezescu & A. M. Holban (Eds.), Quality control in the beverage industry (pp. 225–258). Academic Press (2019). https://doi.org/10.1016/B978-0-12-816681-9.00007-
3. Federación Nacional de Cafeteros de Colombia, *Informe del gerente general al 93 Congreso Nacional de Cafeteros.* Federación Nacional de Cafeteros de Colombia. (2024a). https://www.federaciondecafeteros.org/
4. Federación Nacional de Cafeteros de Colombia, *Dirección de Investigaciones Económicas, Informe mensual de exportaciones: diciembre 2024 [Report]*. Federación Nacional de Cafeteros de Colombia. (2024b). https://federaciondecafeteros.org/wp/informemensualdeexporaciones/
5. K.E. Motato-Rocha, V. González-Montero, M.O. Román-Morales, Identification of microorganisms in wet coffee fermentation Coffea arabica Var Catimor and Castillo in Jardín, Antioquia-Colombia, using culture-dependent methods. Vitae. **31**(1), 1–11 (2024). https://doi.org/10.17533/udea.vitae.v31n1a351373
6. M.A. Díaz Manrique, C.A. Córdoba Vargas, Estudio de la estructura del agroecosistema cafetero mediante el diagrama de ciclos causales: Estudio de caso (Cundinamarca, Colombia). *Ager: Revista de Estudios sobre Despoblación y Desarrollo Rural,* **28,** 135–160 (2020). https://doi.org/10.4422/ager.2019.08
7. M. Montoya Ramírez, Creación de cultura generadora de valor agregado en la actividad cafetera. Gestión Región. **23**, 83–102 (2017). https://revistas.ucp.edu.co/index.php/gestionyregion/article/view/122
8. V.M. Aristizábal, M.A. Chacón, C.A. Cardona, The biorefinery concept for the industrial valorization of coffee processing by-products. In M. A. Farah (Ed.), *Handbook of coffee processing by-products: Sustainable applications* (pp. 61–88). Elsevier (2017). https://doi.org/10.1016/B978-0-12-811290-8.00003-7
9. M. Haile, W.H. Kang, The harvest and post-harvest management practices' impact on coffee quality. In D. T. Castanheira (Ed.), *Coffee: Production and research*. IntechOpen (2019). https://doi.org/10.5772/intechopen.89224
10. S.R. Evangelista, P. Miguel, M.G. da Cordeiro, C.S. Silva, C.F. Pinheiro, A. C. M., R.F. Schwan, Inoculation of starter cultures in a semi-dry coffee (*Coffea arabica*) fermentation process. Food Microbiol. **44**, 87–95 (2014). https://doi.org/10.1016/j.fm.2014.05.013
11. S. Al-Ghamdi, B. Alfalfi, W. Elamin, M.A. Lateef, Advancements in coffee manufacturing: From dehydration techniques to quality control. Food Eng. Rev. **16**, 513–539 (2024). https://doi.org/10.1007/s12393-024-09383-5
12. M.A. Karim, F. Wijayanti, A. Sudaryanto, Comparative studies of coffee processing methods for decision making in appropriate technology implementation. In AIP Conference Proceedings (Vol. 2114, No. 1, p. 020015). AIP Publishing (2019)
13. D. Athinuwat, A. Klomchit, K. Phonwong, P. Nachalaem, R. Thammakulrajang, S. Brooks, Assessing the Impact of Yeast Fermentation in Dry Processing on Coffee Quality from Coffea arabica cv. Caltimor, C. arabica cv. Bourbon and C. canephora cv. Robusta. Trends Sci. **21**(10), 8132 (2024). https://doi.org/10.48048/tis.2024.8132
14. A.P.P. Bressani, S.J. Martinez, A.B.I. Sarmento, F.M. Borém, R.F. Schwan, Influence of yeast inoculation on the quality of fermented coffee (*Coffea arabica* var. *Mundo Novo*) processed by natural and pulped natural processes. Int. J. Food Microbiol. **343**, 109107 (2021). https://doi.org/10.1016/j.ijfoodmicro.2021.109107
15. I. Prakash, S.R. Shankar, H.P. Sneha, P. Kumar, H. Om, K. Basavaraj, P.S. Murthy, Metabolomics and volatile fingerprint of yeast fermented robusta coffee: A value added coffee. LWT. **154**, 112717 (2022). https://doi.org/10.1016/j.lwt.2021.112717
16. Specialty Coffee Association, (s. f.). Specialty Coffee Association. https://sca.coffee/
17. G.S. Giomo, F.M. Borém, Specialty coffees in Brazil: Choice for quality. Informe Agropecuário - Empresa de Pesquisa Agropecuária de Minas Gerais. **32**(263), 7–13 (2011)
18. L.J.C. Ferreira, M. Gomes, de S. Oliveira, L. M., L.D. Santos, Coffee fermentation process: A review. Food Res. Int. **169**, 112793 (2023). https://doi.org/10.1016/j.foodres.2023.112793
19. A.M. Polanía Rivera, J. López Silva, L.S. Torres-Valenzuela, J.L. Plaza Dorado, Development of Starter Inoculum for Controlled Arabica Coffee Fermentation Using Coffee By-Products (Pulp and Mucilage Broth), Yeast, and Lactic Acid Bacteria. Fermentation. **10**(10), 516 (2024). https://doi.org/10.3390/FERMENTATION10100516/S1
20. A.M. Polanía-Rivera, J. López-Silva, L. Torres-Valenzuela, J.L. Plaza-Dorado, From Waste to Taste: Coffee By-Products as Starter Cultures for Sustainable Fermentation and Improved Coffee Quality. Sustainability. **16**(23), 10763 (2024). https://doi.org/10.3390/su162310763
21. L. Duque-Buitrago, K. Calderón-Gaviria, L. Torres-Valenzuela, M. Sánchez-Tamayo, J. Plaza-Dorado, Modulating Coffee Fermentation Quality Using Microbial Inoculums from Coffee By-Products for Sustainable Practices in Smallholder Coffee Production. Sustainability. **17**(5), 1781 (2025). https://doi.org/10.3390/su17051781
22. X. Shen, Q. Wang, H. Wang, G. Fang, Y. Li, J. Zhang, K. Liu, Microbial characteristics and functions in coffee fermentation: A review. Fermentation. **11**(1), 5 (2025). https://doi.org/10.3390/fermentation11010005
23. C. López-Rodríguez, C. Verheecke-Vaessen, C. Strub, A. Fontana, T. Guehi, S. Schorr-Galindo, A. Medina, Resilience to Climate Change by Biocontrol Yeasts Against Ochratoxin A Production in Robusta Coffee. Toxins. **17**(3), 110 (2025). https://doi.org/10.3390/toxins17030110
24. M.L. Souza, L.S. Ribeiro, M.G.C.P. Miguel, L.R. Batista, R.F. Schwan, F.H. Medeiros, C.F. Silva, Yeasts prevent ochratoxin A contamination in coffee by 121 displacing Aspergillus carbonarius. Biol. Control. **155**, 104512 (2021). https://doi.org/10.1016/j.biocontrol.2020.104512
25. A.S. Vale, G. Balla, L.R.S. Rodrigues, D.P. Neto, de C. Soccol, C. R., G.V. de Pereira, M. Understanding the effects of self-induced

anaerobic fermentation on coffee beans quality: Microbiological, metabolic, and sensory studies. Foods. **12**(1), 37 (2023). https://doi.org/10.3390/foods12010037
26. A. González, Obtención y evaluación de polisacáridos de una bacteria ácido láctica para uso como aditivo alimentario [Master thesis, Universidad del Valle] (2017). https://opac.univalle.edu.co/cgi-olib/
27. G.A. Bolívar Escobar, Cepario de Microorganismos y Hongos Filamentosos de la Universidad del Valle. v2.2. Universidad del Valle. Dataset/Occurrence (2023). https://doi.org/10.15472/aj08hw
28. D. Briceño-Vélez, Efecto de las bacterias lácticas microencapsuladas en la calidad microbiológica y en las propiedades fisicoquímicas de salchichas cocidas [Master's thesis, Universidad del Valle] (2020).
29. Oxoid, (2020, febrero). McFarland Equivalence Turbidity Standards (Instrucciones de uso AT00477A). https://www.oxoid.com/ifu
30. Criollo Núñez J, Ramírez-Toro C, Bolívar G, Sandoval AP, Lozano Tovar MD. Effect of microencapsulated Pichia kudriavzevii inoculum on fermentation and sensory quality of CCN51 cocoa genotype. J. Sci. Food Agric. 103(5), 2425–2435 (2023). https://doi.org/10.1002/jsfa.12433
31. M.A. Samaniego, Evaluación de maceración carbónica y adición de levaduras (*Saccharomyces cerevisiae*) durante el lavado de café Geisha (*Coffea arabica*) (Doctoral dissertation, Zamorano: Escuela Agrícola Panamericana, 2019.) (2019)
32. A.G. Herrera, Coliforms. In J. F. T. Spencer & A. L. de Ragout Spencer (Eds.), *Food microbiology protocols* (Methods in Biotechnology, Vol. 14, pp. 29–36). Humana Press (2001). https://doi.org/10.1385/1-59259-029-2:29
33. AOAC, *Official methods of analysis of AOAC International*, 17th edn. (AOAC International, 2000)
34. G.L. Miller, Use of dinitrosalicylic acid reagent for determination of reducing sugar. Anal. Chem. **31**(3), 426–428 (1959). https://doi.org/10.1021/ac60147a030
35. ICONTEC, *NTC 4623: Green coffee. Determination of titratable acidity*, 1st edn. (Colombian Institute of Technical Standards and Certification, 1999)
36. T. Bilenler, I. Karabulut, K. Candogan, Effects of encapsulated starter cultures on microbial and physicochemical properties of traditionally produced and heat-treated sausages (sucuks). LWT Food Sci. Technol. 75, 425–433 (2017). https://doi.org/10.1016/j.lwt.2016.09.003
37. J.E. Bravo-Buitrago, H.J. Ciro-Velásquez, C.J. Márquez-Cardozo, Study of the physicochemical and mechanical stability of an edible leather of mango (Mangifera indica) and pineapple (Ananas comosus) pulp. Rev. Fac. Nac. Agron. Medellín. 76(1), 10189–10200 (2023). https://doi.org/10.15446/rfnam.v75n3.100730
38. P.M.M. Martins, N.N. Batista, L.D. Santos, D.R. Dias, R.F. Schwan, Microencapsulation of epiphytic coffee yeasts by spray drying using different wall materials: Implementation in coffee medium. Int. J. Food Microbiol. **379**, 109839 (2022). https://doi.org/10.1016/j.ijfoodmicro.2022.109839
39. P.M.M. Martins, L.S. Ribeiro, M.G.C.P. Miguel, S.R. Evangelista, R.F. Schwan, Production of coffee (Coffea arabica) inoculated with yeasts: impact on quality. J. Sci. Food. Agric. (2019). https://doi.org/10.1002/jsfa.9820
40. De F. Bruyn, S.J. Zhang, V. Pothakos, J. Torres, C. Lambot, A.V. Moroni, M. Callanan, W. Sybesma, S. Weckx, De L. Vuyst, Exploring the impacts of postharvest processing on the microbiota and metabolite profiles during green coffee bean production. Appl. Environ. Microbiol. **83**(1), e02398–e02316 (2016). https://doi.org/10.1128/AEM.02398-16
41. H. Elhalis, J. Cox, J. Zhao, Coffee fermentation: Expedition from traditional to controlled process and perspectives for industrialization. Appl. Food Res. **3**(1), 100253 (2023). https://doi.org/10.1016/j.afres.2022.100253
42. A.M. Sánchez-Riaño, C. Vega-Oliveros, W.L. Ladino-Garzón, A.F. Bahamón-Monje, N. Gutiérrez-Guzmán, C.M. Amorocho-Cruz, Effects of cherries sanitization methods and fermentation times on quality parameters of coffee beans. Heliyon. **10**(13), e033508 (2024) https://doi.org/10.1016/j.heliyon.2024.e033508
43. A. Díaz, M. Silva, J. Dávila, Relación entre las buenas prácticas de higiene y la ocurrencia de ocratoxina A en café (*Coffea arabica* L.) orgánico de las principales zonas cafetaleras del Perú. Scientia Agropecuaria. **9**(2), 177–187 (2018). https://doi.org/10.17268/sci.agropecu.2018.02.02
44. G. Blandón Castaño, M.T. Dávila Arias, N. Rodríguez Valencia, Caracterización microbiológica y fisicoquímica de la pulpa de café sola y con mucílago, en proceso de lombricompostaje. Revista Cenicafé. **50**(1), 5–23 (1999). https://www.cenicafe.org/es/publications/arc050%2801%29005-023.pdf
45. C. Carranza, R. León, N. Falcón, A. Neumann, C. Kromm, Characterization and distribution of potentially avian pathogenic *Escherichia coli* isolates from broilers in Peru. Revista de Investigaciones Veterinarias del. Perú (RIVEP). **23**(2), 209–219 (2012)
46. A. Lal, N. Cheeptham, *Eosin-Methylene Blue Agar Plates Protocol* (American Society for Microbiology, 2007)
47. N.M. Córdoba Castro, J.E. Guerrero Fajardo, Caracterización de los procesos tradicionales de fermentación de café en el departamento de Nariño. Biotecnología en el Sect. Agropecuario y Agroindustrial (BSAA). **14**(2), 75–83 (2016). https://revistas.unicauca.edu.co/index.php/biotecnologia/article/view/480
48. G.I. Puerta, Fundamentos del proceso de fermentación en el beneficio del café (2013)
49. J.M.C. Palumbo, P.M.M. Martins, L.G.A. Salvio, N.N. Batista, L.S. Ribeiro, F.M. Borém, D.R. Dias, R.F. Schwan, Impact of different fermentation times on the microbiological, chemical, and sensorial profile of coffees processed by selfinduced anaerobiosis fermentation. Brazilian J. Microbiol. **55**(3), 2253–2266 (2024). https://doi.org/10.1007/S42770-024-01370-6/FIGURES/4
50. G.I. Puerta, Fermentación controlada del café: Tecnología para agregar valor a la calidad (2015). https://www.cenicafe.org
51. J. Smith, A laboratory manual customized for Morton University: Microbiology. Morton Publishing Company. Recuperado de (2020) https://www.morton-pub.com/wpcontent/uploads/2020/12/customlab_cover_wpages_micro.pdf
52. A.M. Ebabhi, A.A. Adekunle, W.O. Okunowo, A.A. Osuntoki, Isolation and characterization of yeast strains from local food crops. J. Yeast Fungal Res. **4**(4), 38–43 (2013). https://doi.org/10.5897/JYFR2013.0112
53. D. Stanley, A. Bandara, S. Fraser, P.J. Chambers, G.A. Stanley, The ethanol stress response and ethanol tolerance of Saccharomyces cerevisiae. J. Appl. Microbiol. **109**(1), 13–24 (2010). https://doi.org/10.1111/j.1365-2672.2009.04657.x
54. J. Antonio, P. Agüera, Efecto de la sobrepresión de CO_2 endógeno sobre el proteoma de levaduras vínicas durante la segunda fermentación en la elaboración de cava [Master's thesis, Universidad de Córdoba]. *Helvia* (2020). http://helvia.uco.es/xmlui/handle/10396/20347
55. J.O. Westman, C.J. Franzén, Current progress in high cell density yeast bioprocesses for bioethanol production. Biotechnol. J. **10**(8), 1185–1195 (2015). https://doi.org/10.1002/biot.201400581
56. S. Kaur, K. Kaur, B. Bhushan, M. Kaur, M. Hans, Inoculum size and age studies on single and mixed strain fermentation of grape juice. J. Pure Appl. Microbiol. **14**(3), 2137–2145 (2020). https://doi.org/10.22207/JPAM.14.3.54
57. B.L. da Silva, P.V. Pereira, L.D. Bertoli, D.L. Silveira, N.N. Batista, P.F. Pinheiro, J. Carneiro, de S. Schwan, R.F. Silva, S. de Coelho, J. M., P.C. Bernardes, Fermentation of Coffea canephora inoculated with yeasts: Microbiological, chemical, and sensory

characteristics. Food Microbiol. **98**, 103786 (2021). https://doi.org/10.1016/j.fm.2021.103786
58. P.E.G. Paixão, M.V.S. Couto, N.C. Sousa, H.A. Abe, J.A.R. Dias, J.O. Meneses, F.S. Cunha, J.L.P. Mouriño, M.L. Martins, R.Y. Fujimoto, In vitro selection of autochthonous lactic acid bacterium from clownfish Amphiprion ocellaris. Aquac. Res. **00**, 1–4 (2019). https://doi.org/10.1111/are.14396
59. Gutierrez-Sarmiento W, Tosvilla-Coutiño ML, Ventura-Canseco LMC. Evaluation of extrinsic factors on the growth of *Lactobacillus plantarum* and its lactic acid production in a stirred-tank reactor. Academia Journals. 7(1), (2015). . http://repositoriodigital.tuxtla.tecnm.mx/xmlui/handle/123456789/3138
60. G.X.R. Costa, L.C.F. Silva, L.M. Oliveira, L.D. Santos, Microbiota of Arabica coffee: Insights from soil to fruit. World J. Microbiol. Biotechnol. **40**(10), 308 (2024). https://doi.org/10.1007/s11274-024-04110-y
61. G.V. Pereira, de M. Mello Sampaio, V. Wiele, N. da Silva Vale, A. de Carvalho Neto, D.P. Souza, A. de Ruiz, I.R. Rogez, H., C.R. Soccol, How yeast has transformed the coffee market by creating new flavors and aromas through modern post-harvest fermentation systems. Trends Food Sci. Technol. 151, 104641 (2024). https://doi.org/10.1016/J.TIFS.2024.104641
62. G.I. Puerta, Factores, procesos y controles en la fermentación del cafe (2012). https://www.cenicafe.org
63. A.E. Peñuela-Martínez, J.F. García-Duque, J.R. Sanz-Uribe, Characterization of fermentations with controlled temperature with three varieties of coffee (Coffea arabica L). Fermentation. **9**(11), 976 (2023). https://doi.org/10.3390/fermentation9110976
64. L. Rosenboim, The importance of fungal enzymes in industry and biotechnology. Fungal Genomics Biology. **14**(4), 267 (2024). https://doi.org/10.35248/2165-8056.24.14.267
65. I. Hajok, A. Kowalska, A. Piekut, M. Ćwieląg-Drabek, A risk assessment of dietary exposure to ochratoxin A for the Polish population. Food Chem. **284**, 264–269 (2019)
66. N. Yazdanfar, T. Mahmudiono, Y. Fakhri, A.H. Mahvi, P. Sadighara, A.A. Mohammadi, M. Yousefi, Concentration of ochratoxin A in coffee products and probabilistic health risk assessment. Arab. J. Chem. **15**(12), 104376 (2022). https://doi.org/10.1016/j.arabjc.2022.104376
67. A. Valero, S. Marín, A.J. Ramos, V. Sanchis, Effect of yeast and lactic acid bacteria on growth and ochratoxin A production by Aspergillus carbonarius in grape must. Int. J. Food Microbiol. **113**(3), 281–288 (2007). https://pubmed.ncbi.nlm.nih.gov/31785405/
68. Y. Wu, C. Ji, Y. Jiang, H. Hu, T. Yu, F. Yan, Mechanisms of *Meyerozyma caribbica* isolated from Tibetan soil to inhibit Aspergillus ochraceus on grapes. Postharvest Biol. Technol. **210**, 112797 (2024). https://doi.org/10.1016/j.postharvbio.2024.112797
69. M.F. Salazar-Hernández, J.A. Lona-Ramírez, C. Rivera-Domínguez, V.M. Sánchez-Núñez, H. Rosales-Bravo, N.A. Caudillo-Ortega, Evaluación de compuestos químicos de la variedad Típica de Coffea arabica durante el proceso del tostado artesanal. Investigación y Ciencia. **29**(81), 32–39 (2020). https://www.redalyc.org/articulo.oa?id=67466172004
70. R.M. Niemi, J. Mentu, A. Siitonen, S.I. Niemelä, Confirmation of Escherichia coli and its distinction from Klebsiella species by gas and indole formation at 44 and 115 44.5 degrees C. J. Appl. Microbiol. **95**(6), 1242–1249 (2003). https://doi.org/10.1046/j.1365-2672.2003.02125.x
71. A.V.U. Braga, M.A. Miranda, H. Aoyama, F.L. Schmidt, Study on coffee quality improvement by self-induced anaerobic fermentation: Microbial diversity and enzymatic activity. Food Res. Int. **165**, 112528 (2023). https://doi.org/10.1016/j.foodres.2023.112528
72. M. Olstorpe, J. Schnürer, V. Passoth, Growth inhibition of various Enterobacteriaceae species by the yeast Hansenula anomala during storage of moist cereal grain. Appl. Environ. Microbiol. **78**(1), 292–294 (2012). https://doi.org/10.1128/AEM.06024-11
73. A.S. Vale, C.M.T. Pereira, J.D. Lindner, L.R.S. Rodrigues, E. Kadri, N.K. Pagnoncelli, M.G.B. Brar, S. K., de G.V. Melo Pereira, Exploring Microbial Influence on Flavor Development during Coffee Processing in Humid Subtropical Climate through Metagenetic-Metabolomics Analysis. Foods. **13**(12), 1871 (2024). https://doi.org/10.3390/foods13121871
74. L. Jespersen, D.S. Nielsen, S. Hønholt, M. Jakobsen, Occurrence and diversity of yeasts involved in fermentation of West African cocoa beans. FEMS Yeast Res. **5**(4), 441–453 (2005). https://doi.org/10.1016/j.femsyr.2004.11.002
75. C.F. Silva, L.R. Batista, L.M. Abreu, E.S. Dias, R.F. Schwan, Succession of bacterial and fungal communities during natural coffee (Coffea arabica) fermentation. Food Microbiol. **25**(8), 951–957 (2008). https://doi.org/10.1016/j.fm.2008.07.003
76. M. Ginovart, C. Prats, X. Portell, M. Silbert, Analysis of the effect of inoculum characteristics on the first stages of a growing yeast population in beer fermentations by means of an individual-based model. J. Ind. Microbiol. Biotechnol. **38**(1), 153–165 (2011). https://doi.org/10.1007/s10295-010-0840-4
77. G.V. Pereira, de M. Carvalho Neto, D.P. Magalhães Júnior, A.I. Vásquez, Z.S. Medeiros, A.B.P. Vandenberghe, L. P. S., C.R. Soccol, Exploring the impacts of postharvest processing on the aroma formation of coffee beans – A review. Food Chem. **272**, 441–452 (2019). https://doi.org/10.1016/j.foodchem.2018.08.061
78. E.G. Coelho, P.L.L. Bertarini, M.S. Gomes, L.R. Amaral, M.F. Zotarelli, L.D. Santos, R.C. Santana, Physicochemical and sensory properties of Arabica coffee beans of Arara cv. dried using different methods. Foods. **13**(5), 642 (2024). https://doi.org/10.3390/foods13050642
79. L.W. Lee, M.W. Cheong, P. Curran, B. Yu, S.Q. Liu, Coffee fermentation and flavor – An intricate and delicate relationship. Food Chem. **185**, 182–191 (2015). https://doi.org/10.1016/j.foodchem.2015.03.124
80. G. Galarza, J.G. Figueroa, Volatile compound characterization of coffee (*Coffea arabica*) processed at different fermentation times using SPME–GC–MS. Molecules **27**(6), 2004 (2022). https://doi.org/10.3390/molecules27062004
81. A.P.P. Bressani, S.J. Martinez, S.R. Evangelista, D.R. Dias, R.F. Schwan, Characteristics of fermented coffee inoculated with yeast starter cultures using different inoculation methods. LWT. **92**, 212–219 (2018)
82. L. Holguín-Sterling, B. Pedraza-Claros, R. Pérez-Salinas, A. Ortiz, L. Navarro-Escalante, C.E. Góngora, Physical-Chemical and Metataxonomic Characterization of the Microbial Communities Present During the Fermentation of Three Varieties of Coffee from Colombia and Their Sensory Qualities. Agriculture. **13**(10), 1980 (2023) https://doi.org/10.3390/agriculture13101980
83. K. Nakyinsige, F. Mugerwa, A. Tabula, D. Mugampoza, M. Matovu, M. Bamuwamye, The influence of spontaneous microbial fermentation isolates on physicochemical properties and cup quality of wet processed Arabica coffee (Coffea arabica). LWT - Food Sci. Technol. **216**, 117343 (2025). https://doi.org/10.1016/j.lwt.2025.117343
84. N.T. Fadai, J. Melrose, C.P. Please, A. Shulman, Van R.A. Gorder, A heat and mass transfer study of coffee bean roasting. Int. J. Heat Mass Transf. **104**, 787–799 (2017). https://doi.org/10.1016/j.ijheatmasstransfer.2016.08.083
85. T. pls cite, A. Joët, F. Laffargue, S. Descroix, B. Doulbeau, A. Bertrand, de S. Kochko, Dussert, Influence of environmental factors, wet processing and their interactions on the biochemical composition of green Arabica coffee beans. Food Chem. **118**(3), 693–701 (2010). https://doi.org/10.1016/j.foodchem.2009.05.048
86. G.V. Pereira, de M. Neto, E. Soccol, V.T. Medeiros, A.B.P. Woiciechowski, A. L., C.R. Soccol, Conducting starter

culture-controlled fermentations of coffee beans during on-farm wet processing: Growth, metabolic analyses and sensorial 117 effects. Food Res. Int. **75**, 348–356 (2015). https://doi.org/10.1016/j.foodres.2015.06.027
87. R. Bollen, O. Rojo-Poveda, B.N. Katshela et al., The impact of coffee genotype and processing method on the sensory and metabolite profiles of Robusta coffee (*Coffea canephora*). Eur. Food Res. Technol. **251**, 1171–1190 (2025). https://doi.org/10.1007/s00217-025-04696-z
88. H. Lin, E. Tello, C.T. Simons, D.G. Peterson, Identification of Non-Volatile Compounds Generated during Storage That Impact Flavor Stability of Ready-toDrink Coffee. Molecules. **27**(7), 2120 (2022). https://doi.org/10.3390/molecules27072120
89. D. Mardhatilah, F. Padama, Ngatirah, Development of sustainable arabica coffee fermentation using yeast starter. IOP Conf. Ser. Earth Environ. Sci. **1241**(1), 012078 (2023). https://doi.org/10.1088/1755-1315/1241/1/012078
90. P.A. Silva, V.M. Rabelo, J.M.R. Calixto, P. de Coelho O, Quality assessment of coffee grown in Campos Gerais, Minas Gerais State, Brazil. Acta Scientiarum Technol. **36**(4), 739–744 (2014). https://doi.org/10.4025/actasciencitechnol.v36i4.19765
91. V. Tirado-Kulieva, C. Quijano-Jara, H. Avila-George, W. Castro, Predicting the evolution of pH and total soluble solids during coffee fermentation using nearinfrared spectroscopy coupled with chemometrics. Curr. Res. Food Sci. **9**, 100788 (2024). https://doi.org/10.1016/j.crfs.2024.100788
92. A.P.P. Bressani, S.J. Martinez, A.B.I. Sarmento, F.M. Borém, R.F. Schwan, Organic acids produced during fermentation and sensory perception in specialty coffee using yeast starter culture. Food Res. Int. **128**, 108773 (2020)
93. Y. Liu, W. Yuan, Y. Lu, S.Q. Liu, Biotransformation of spent coffee grounds by fermentation with monocultures of Saccharomyces cerevisiae and Lachancea thermotolerans aided by yeast extracts. LWT. **138**, 110751 (2021). https://doi.org/10.1016/j.lwt.2020.110751
94. H. Tieghi, L.A. Pereira, G.S. Viana, A. Katchborian-Neto, D.B. Santana, R.L. Mincato, D.F. Dias, D.A. Chagas-Paula, M.G. Soares, de W.G. Araújo, P.C.P. Bueno, Effects of geographical origin and post-harvesting processing on the bioactive compounds and sensory quality of Brazilian specialty coffee beans. Food Res. Int. **186**, 114346 (2024). https://doi.org/10.1016/J.FOODRES.2024.114346
95. G.I. Puerta, Composición química de una taza de café (Avance Técnico No. 414). Centro Nacional de Investigaciones de Café (Cenicafé) (2011). https://biblioteca.cenicafe.org/bitstream/10778/340/1/avt0414.pdf
96. W. Sangpimpa, P. Srichan, T. Jannu et al., Influence of roasting technique on physicochemical properties, volatile compounds, and flavor profile of robusta (*Coffea canephora*) coffee beans. Eur. Food Res. Technol. (2025). https://doi.org/10.1007/s00217-025-04866-z
97. L.F. Echeverri-Giraldo, O. Pérez, V.T. Arboleda, C. Vargas, L.J. Gutiérrez, L.C. Imbachí Quinchua, Content of acidic compounds in the bean of *Coffea arabica* L., produced in the Department of Cesar (Colombia), and its relationship with the sensorial attribute of acidity. Separations. **11**(2), 52 (2024). https://doi.org/10.3390/separations11020052
98. M.M.B. Simmer, C.A. Filete, G.L. Marotto et al., Combined effects of harvest time and processing on the chemical composition and sensory quality of Arabica coffee. Eur. Food Res. Technol. (2025). https://doi.org/10.1007/s00217-025-04870-3
99. I. Trandafir, V. Nour, M.E. Ionica, Antioxidant capacity, phenolic acids and caffeine contents of some commercial coffees available on the Romanian market. Arch. Latinoam. Nutr. **63**(1), 87–94 (2013)
100. H. Wu, P. Lu, Z. Liu, J. Sharif-Rad, H.A.R. Suleria, Impact of roasting on the phenolic and volatile compounds in coffee beans. Food Sci. Nutr. **10**(7), 2408–2425 (2022). https://doi.org/10.1002/fsn3.2849
101. Y. Tan, H. Wu, L. Shi, C. Barrow, F.R. Dunshea, H.A.R. Suleria, Impacts of fermentation on the phenolic composition, antioxidant potential, and volatile compounds profile of commercially roasted coffee beans. Fermentation. **9**(10), 918 (2023). https://doi.org/10.3390/fermentation9100918
102. I. Hečimović, A. Belščak-Cvitanović, D. Horžić, D. Komes, Comparative study of polyphenols and caffeine in different coffee varieties affected by the degree of roasting. Food Chem. **129**(3), 991–1000 (2011). https://doi.org/10.1016/j.foodchem.2011.05.059
103. L.M. Pérez-Hernández, K. Chávez-Quiroz, L.A. Medina-Juárez, N. Gámez Meza, Phenolic Characterization, Melanoidins, and Antioxidant Activity of Some Commercial Coffees from Coffea arabica and Coffea canephora. J. Mex. Chem. Soc. **56**(4), 430–435 (2012)
104. C. Huaccha-Herrera, E. Ordoñez-Gómez, J.E. Villanueva-Tiburcio, Effect of the roasting process on total polyphenol content, antioxidant activity, and sensory evaluation of coffee produced in Peru. Ciencia Tecnología Agropecuaria. **25**(3), 3508 (2024). https://doi.org/10.21930/rcta.vol25_num3_art.3508
105. R. Vatinno, A. Aresta, C.G. Zambonin, F. Palmisano, Determination of Ochratoxin A in green coffee beans by solid-phase microextraction and liquid chromatography with fluorescence detection. J. Chromatogr. A **1187**(1–2), 145–150 (2008). https://doi.org/10.1016/j.chroma.2008.02.020
106. European Commission. (2023). Commission Regulation (EU) 2023/915 of 25 April 2023 on maximum levels for certain contaminants in food and repealing Regulation (EC) No 1881/2006. Official Journal of the European Union, L 119, 103–157. http://data.europa.eu/eli/reg/2023/915/oj
107. M. Lobeau, De S. Saeger, L. Sibanda, I. Barna-Vetró, Van C. Peteghem, Development of a new clean-up tandem assay column for the detection of ochratoxin A in roasted coffee. Anal. Chim. Acta. **538**(1–2), 57–61 (2005)
108. E.B. Estrada-Bahena, R. Salazar, M. Ramírez, M.E. Moreno-Godínez, J. Jiménez-Hernández, Y. Romero-Ramírez, P. Alvarez-Fitz, Influence of water activity on physical properties, fungal growth, and ochratoxin A production in dry cherries and green-coffee beans. J. Food Process. Preserv. **46**(2), e16226 (2022)

Publisher's Note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.