

Microbial Succession in Arabica Coffee Fermented Using a Carbonic Maceration-based Ohmic Technology Fermenter

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Editor: Samuel Pereira de Carvalho

Received in November 14, 2025 and approved in May 7, 2026

ABSTRACT

Coffee fermentation under carbonic-maceration-based closed conditions with self-induced anaerobiosis is sensitive to temperature. This study evaluated physicochemical trajectories and culturable bacterial succession during 24–240 h of whole-cherry Arabica fermentation at 31 and 36 °C in an ohmic-controlled sealed fermenter. Fermentation liquid was analysed for pH, °Brix, total dissolved solids (TDS), culturable bacterial counts, Gram characteristics, presumptive genus-level identification, and MALDI-TOF MS identification of selected isolates. pH decreased rapidly from 24 to 48 h at both temperatures and then remained relatively stable. TDS remained stable at 31 °C (2.41–2.60%) but increased at 36 °C to 3.26 ± 0.10% at 240 h, whereas °Brix increased over time and was higher overall at 36 °C. Descriptive culture-dependent observations showed persistent Gram-negative dominance at 31 °C, with presumptive shifts from *Klebsiella* to *Acinetobacter* and subsequently to *Pseudomonas* and *Alcaligenes*. At 36 °C, Gram-positive bacteria contributed more during early to mid-fermentation, with presumptive *Lactobacillus* at 24–48 h and *Bacillus* at 72–120 h. MALDI-TOF MS identified selected isolates obtained at 72 h as *Bacillus subtilis*, *B. amyloliquefaciens*, *B. vallismortis*, and *B. idriensis*. The results indicate distinct temperature-associated physicochemical and culturable bacterial trajectories, with 72–96 h at 36 °C representing a potential transition period.

Key words: Arabica coffee; carbonic maceration; culturable bacteria; fermentation temperature; ohmic heating; self-induced anaerobiosis.

1 INTRODUCTION

Coffee fermentation can aid in maintaining consistent quality and differentiating flavours, especially considering climate and post-harvest uncertainty. During fermentation, a community of microbes, such as yeast, lactic acid bacteria (LAB), and acetic acid bacteria (AAB), mediates the transformation of sensory precursors through metabolic pathways that shape the acidity, body, and volatile profile of coffee (Elhalis et al., 2020; Haile & Kang, 2019). Chemical substrates, such as lipids, proteins, and precursors, modulate the formation of flavour and aroma compounds during fermentation. Under wet-processing conditions, microbial dynamics depend on process parameters such as fermentation time, temperature, and oxygen availability. Changes in these parameters can alter microbial succession, metabolite formation, and cup quality (De Bruyn et al., 2017; Peñuela-Martínez et al., 2015; Zhang et al., 2019a). Carbonic maceration, adapted from enology, is a targeted approach for modulating the fermentation ecology. Recent studies have demonstrated that carbonic maceration alters bacterial communities and enhances the sensory attributes of coffee (Da Silva et al., 2023). Temperature and time control after harvest increases the Specialty Coffee Association score and preserves the sensory profile.

In coffee post-harvest practices, the concept of carbonic maceration does not always require CO₂ flushing at the

beginning of the process, as several studies have shown that the fermentation of whole coffee cherries in closed tanks/bioreactors can gradually create CO₂-rich anaerobic conditions (self-induced anaerobic fermentation/SIAF) through the mechanism of O₂ consumption and CO₂ production by microbial metabolic activity during fermentation, so that the system progressively shifts from initial conditions that still contain oxygen to a more anaerobic/CO₂-dominant atmosphere. This approach has been used as a framework to distinguish “closed” fermentation (SIAF) from “open/semi-anaerobic” fermentation in coffee cherries and depulped coffee cherries. It has been reported to use a closure design (e.g. screw cap with airlock) that allows CO₂ to escape but prevents outside air from entering—thus operationally aligning with the definition of a closed fermentation process that produces an internal CO₂-rich atmosphere without external injection, and is relevant to the position as a semi/partial carbonic maceration variant based on endogenous CO₂ formation in cherries (Da Silva Vale et al., 2023).

However, precise temperature control is a prerequisite for replicating sensory profiles, especially because climate change alters plant physiology and the composition of the metabolic substrates of coffee fruits. In principle, ohmic heating generates heat volumetrically through electrical resistance throughout the mass of the material, so that heating occurs more quickly and more uniformly and reduces the temperature

gradient that commonly occurs in conventional heating based on heat transfer from the walls (e.g., water bath/jacket), which in fermentation can trigger variations in microbial kinetics between batches (Sakr & Liu, 2014)

The advantages of ohmic heating-based fermenters include rapid and uniform volumetric heating, high hygiene, and real-time monitoring of temperature. These features enable the stabilisation of biochemical fermentation pathways. Previous studies have demonstrated that ohmic fermentation can reduce the acidity of Arabica beans, providing opportunities for implementing process interventions such as carbonic maceration (Mustafa *et al.*, 2024; Reta *et al.*, 2017). Thus, focusing on microbial succession and its relationship with pH trajectory, °Brix, and total dissolved solids (TDS) in carbonic maceration using an ohmic fermenter can provide useful insights for the post-harvest science and specialty coffee industry.

The major challenge in developing Arabica coffee fermentation based on carbonic maceration with ohmic heating is controlling the bioprocess pathways, which are highly sensitive to temperature, time, oxygen/CO₂ availability, and the mucilage composition of the coffee cherries. These factors significantly affect the microbial succession and sensory quality. Various studies have revealed that different process parameters can shift the predominance of communities (for example, LAB vs. AAB/gram-negative), modulate key metabolites, and alter cupping quality. Thus, precise instrumentation and standardised protocols, such as pH curves, °Brix, and TDS measurements, are required for replication (Da Silva *et al.*, 2023).

In contrast, carbonic maceration affects the structure of bacterial communities and the sensory attributes of coffee. However, the heterogeneity of microbial responses across terroirs and post-harvest practices highlights the need for a contextual succession map (Da Silva *et al.*, 2023). Previously described culture-dependent and culture-independent approaches present rich patterns of succession. However, translating these findings to the production line is challenging owing to microbiome variability, monitoring costs, and limitations in real-time capabilities (Silva *et al.*, 2008; Todhanakasem *et al.*, 2024). Ohmic heating offers rapid and homogeneous heating to stabilise fermentation dynamics. However, scaling up raises engineering issues, such as electrode design, energy efficiency, and inline sensor integration. Early evidence suggests the potential for reduced acidity and a smooth flavour profile (Reta *et al.*, 2017). To address market pressures and global quality-sustainability policies, precision fermentation, which combines carbonic maceration with ohmic temperature control and metabolite/community monitoring, creates opportunities for the development of adaptive microbial starters (Mustafa *et al.*, 2024).

The fermentation and postharvest processing of Arabica coffee have been mapped in terms of microbial succession and

metabolite changes from coffee cherries through green-bean production (Zhang *et al.*, 2019b). Process-parameterisation studies have further demonstrated that fermentation conditions, including temperature, duration, and oxygen availability, can influence microbial-community dynamics, metabolomic profiles, and cup quality during wet coffee processing (Zhang *et al.*, 2019a). The carbonic maceration approach affects bacterial communities and sensory attributes, indicating the relevance of controlling the fermentation environment to modulate the outcome of the coffee fermentation (Da Silva *et al.*, 2023). Ohmic fermentation innovation also demonstrates technological potential by reducing the acidity of beans, which has implications for the flavour, cleanliness, and stability of the process (Reta *et al.*, 2017).

The natural microbial succession map has been described as moving from the initial predominance of gram-negative bacteria/yeast to the establishment of certain gram-positive bacteria. However, variations in patterns across different terroirs and protocols lead to heterogeneous interpretations (Silva *et al.*, 2008). Recent studies have reported a correlation between community structure and the aroma and chemical attributes of the final product. These findings indicate the need for simultaneous integration of microbial and physicochemical data (Todhanakasem *et al.*, 2024). The currently available literature has not addressed the following domains in ohmic-based carbonic maceration: (i) the effect of two narrowly defined yet applicable temperature set-points (31 °C vs. 36°C) on the trajectories of pH, °Brix, and TDS over 24–240 h; (ii) the effect of temperature on the microbial community succession (the proportion of gram-negative/gram-positive, as well as genus/species composition) over 10 days; (iii) the temporal correspondence between physicochemical trajectories and changes in the culturable bacterial profile. This study addresses these gaps in the literature. This study aimed to examine the effect of fermentation temperature (31 °C versus 36°C) in ohmic-based carbonic maceration on the pH, °Brix, TDS, and culturable bacterial succession during 24–240 h of ohmic-assisted carbonic-maceration-based fermentation of whole Arabica coffee cherries.

This study contributes to the following three main areas: (i) establishment of an integrative theoretical framework of process-microbe-metabolite that links temperature control in carbonic maceration and ohmic heating with pH pathways, °Brix, TDS, and community succession (gram-negative/gram-positive), enabling precision fermentation; (ii) provision of the basis for formulating policy/process standards guidelines for producers and quality regulators (such as working temperature windows, duration, and inline monitoring requirements), which is based on process parameters shaping community composition and sensory quality; and (iii) provision of a roadmap for future research on adaptive starter consortia, integrated metabolomic sensory analysis, and process

marker-based control algorithms. The empirical foundation is robust, and processing parameters have been reported to alter community dynamics, metabolome, and cup quality during the wet processing of Arabica coffee (De Bruyn et al., 2017; Zhang et al., 2019a). Carbonic maceration specifically alters bacterial structure and sensory attributes (Da Silva et al., 2023). The correlation between microbial communities and the aroma and chemical characteristics of the final product indicates the need to integrate microbial-physicochemical data into quality policy recommendations and traceability (Todhanakasem et al., 2024).

2 MATERIAL AND METHODS

2.1 Raw material-fermentation processing

The materials used in this study were Arabica Coffee (Arabica Coffee) beans harvested in August 2023. Fully ripe Arabica coffee was harvested from a coffee plantation in Tanah Toraja, Kaduaja Village, located in the highlands of Sangbua Rodo-Rodo (approximately 1,400 m above sea level). The coffee fruits were transported to Hasanuddin University in Makassar for fermentation. The time between harvest and the start of fermentation was approximately 36 h at 27°C.

Fermentation was conducted in a closed PVC food-grade tube ohmic fermenter (internal diameter 12.7 cm; length

130 cm) supplied with 220 V AC to generate an electric field strength of 1.7 V/cm. After washing to remove dried fruits, defective beans, leaves, sticks, and other impurities, 10 kg of coffee cherries were loaded, the vessel was filled with potable water, and then tightly sealed with a gasketed sanitary clamp lid to limit oxygen exposure and allow the progressive accumulation of fermentation-derived CO₂ in the headspace (self-induced anaerobiosis). The temperature was maintained at 31 °C or 36°C using an Arduino controller, and 20 mL of fermentation liquid was collected via a mid-vessel sampling tap. Liquid collection begins within the first 24 h (Peñuela-Martínez et al., 2025). Sampling was carried out for 240 h or 10 days at 24-hour intervals, namely, 24, 48, 72, 96, 120, 144, 168, 192, 216, and 240 h. Several studies have been conducted on multi-day fermentation (Braga et al., 2023; Buitrago-Zuluaga et al., 2025; Tangjaidee et al., 2025). Fermentation liquid was stored at freezing temperatures for subsequent analysis of pH, °Brix, TDS, microbial counts, gram classification, and microorganism types (Figure 1). Fermentation was stopped, and the coffee was collected for further analyses. The pulp was removed using a pulping machine. The samples were sun-dried for five days. The coffee beans were then sun-dried until their moisture content reached approximately 12%. The hull was removed using a hull-removing machine. The resulting product was a green coffee bean.

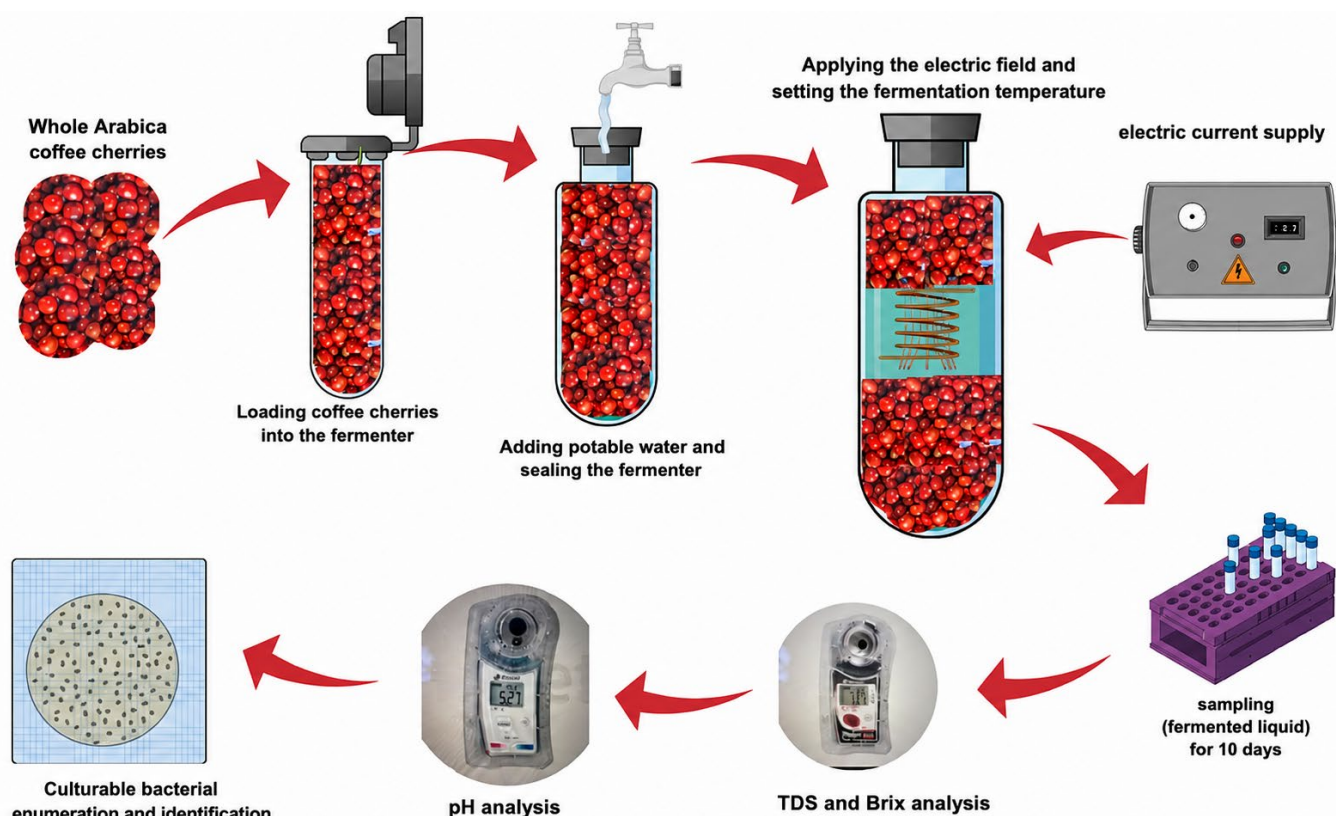


Figure 1: Experimental flow diagram of ohmic-assisted closed fermentation of whole Arabica coffee cherries.

pH, TDS, and °Brix measurements were conducted from 24 to 240 h. Sampling began at 24 h because whole, uncrushed coffee cherries were used, and measurable mass transfer from the fruit matrix into the liquid phase was not expected at the beginning of fermentation. Sampling at 24-h intervals also minimised disturbance of the closed fermentation system (Peñuela-Martínez et al., 2025). The 240-h fermentation period was selected based on previous multi-day coffee-fermentation studies conducted for approximately 192 h to 9 days under closed, semi-anaerobic, or SIAF conditions (Braga et al., 2023; Buitrago-Zuluaga et al., 2025; Peñuela-Martínez et al., 2025; Tangjaidee et al., 2025).

2.2 Preparation of the sample for physicochemical characterization

The pH of the fermentation liquid was measured using a handheld digital pH meter (ATAGO PAL-pH; range, 0–14). The device was calibrated at room temperature using certified pH 4.00 and pH 7.00 buffers (pH 10.00 buffer was used when necessary) according to the manufacturer's procedures. The electrode was rinsed with deionised water and dried slowly before and between the measurements. The samples were homogenised, and the pH was recorded after a stable reading. TDS and °Brix were determined using a digital refractometer (ATAGO PAL-Coffee, BX/TDS) calibrated daily with deionised water (zero point) and periodically verified using a sucrose standard solution. The prism was cleaned and dried, the sample was dripped onto the prism, and the TDS and °Brix values were recorded after stabilisation. The prism was rinsed and dried between each sample. At each sampling time, physicochemical measurements were obtained independently from each of the three fermentors assigned to each temperature treatment. Thus, $n = 3$ represents three independent fermentation replicates rather than repeated instrumental readings from the same fermentation sample. Each sample was measured three times as technical analytical readings, and the average of these readings was used as the single observation for the corresponding fermentor at each sampling time. The results were expressed as mean $\pm$ standard deviation of the three independent fermentors.

2.3 Preparation of the sample for Bacterial enumeration and identification

Fermentation liquid was collected aseptically every 24 h for 10 consecutive days, immediately frozen, and stored at -20°C until analysis. Prior to microbiological analysis, the samples were thawed at room temperature and homogenised. Total culturable bacterial loads were determined using the pour-plate technique on Plate Count Agar (PCA; Merck, Darmstadt, Germany). A sterile diluent (0.9% NaCl, 9.0 mL per tube) was prepared and autoclaved at 121°C for 15 min. Serial decimal dilutions were

performed by transferring 1.0 mL of the homogenised sample into 9.0 mL of diluent (10^{-1}), followed by successive transfers to obtain 10^{-2} and 10^{-3} ; each tube was mixed thoroughly (e.g. vortexed) before the next transfer. For plating, 1.0 mL of the 10^{-3} dilution was dispensed into sterile Petri dishes in duplicate and mixed with 15–20 mL of molten PCA maintained at $45 \pm 1^{\circ}\text{C}$. After solidification, the plates were incubated at 37°C for 24 h. Colonies were enumerated on countable plates and expressed as CFU/mL using the plated volume and dilution factor ($\text{CFU/mL} = \text{colonies} \div [\text{dilution} \times \text{plated volume}]$).

Representative isolates were selected based on colony morphology and frequency on PCA plates, purified by repeated streaking to obtain single colonies, and subjected to Gram staining to classify isolates as Gram-positive or Gram-negative and to assess cell morphology. Biochemical identification was performed using group-specific testing panels. Gram-negative isolates were evaluated using Triple Sugar Iron Agar (TSIA), Sulfide Indole Motility (SIM) medium, Methyl Red–Voges Proskauer (MR–VP) tests, citrate utilization, urease activity, and carbohydrate utilization/fermentation assays (glucose, lactose, sucrose, mannitol, xylose, sorbitol, and rhamnose). Gram-positive cocci were screened on mannitol salt agar and tested for catalase, oxidase, and coagulase activities. Gram-positive bacilli were characterised using carbohydrate utilization/fermentation assays (glucose, lactose, sucrose, mannitol, xylose, sorbitol, and rhamnose). All biochemical tests were incubated at 37°C for 24 h, and reaction profiles were recorded and interpreted using a laboratory identification reference table to assign taxa at the genus level.

2.4 Sample preparation using VITEK mass spectrometry (MS) FlexPrep (software version: 1.0.0.46)

Bacterial isolates obtained from coffee fermentation liquid after 72 h of fermentation at 36°C were purified to single colonies and incubated for 24 h before analysis. One colony from each isolate was prepared using VITEK® Mass Spectrometry (MS) FlexPrep (software version 1.0.0.46) through an automated cell lysis and ribosomal protein extraction process according to the manufacturer's protocol. The protein extract was then spotted onto a MALDI-TOF MS target plate, supplemented with an α -cyano-4-hydroxycinnamic acid (CHCA) matrix, dried at room temperature, and analysed using a VITEK® MS instrument. The resulting mass spectrum was compared with the VITEK® MS database, and species identification was accepted at a confidence level of $\geq 99.9\%$.

2.5 Statistical Analysis

Data for pH, TDS, and °Brix represent the average of measurements and were analysed using a two-way ANOVA, followed by a Tukey post hoc test if there were significant differences.

3 RESULTS

3.1 Physicochemical characteristics of ohmic-assisted carbonic maceration fermentation liquid

3.1.1 pH

The effects of fermentation temperature and duration on the pH of Arabica coffee fermentation liquid are presented in Table 1. The temperature $\times$ fermentation time interaction significantly affected pH ($p < 0.01$). At 31 °C, pH decreased significantly from 3.61 ± 0.01 at 24 h to 3.34 ± 0.01 at 48 h and subsequently remained relatively stable within the range of 3.28–3.37 until 240 h. A similar pattern was observed at 36 °C, where pH decreased from 3.54 ± 0.01 at 24 h to 3.30 ± 0.02 at 48 h and then remained within the range of 3.29–3.36 until 240 h. Thus, the greatest reduction in pH occurred during the first 24–48 h, followed by a relatively stable phase at both temperatures.

Table 1: Effects of fermentation temperature and duration on the pH of coffee fermentation liquid.

Fermentation time (h)	31 °C	36 °C	Mean across temperatures
24	3.61 ± 0.01^a	3.54 ± 0.01^a	3.58 ± 0.04^a
48	3.34 ± 0.01^b	3.30 ± 0.02^b	3.32 ± 0.03^{bc}
72	3.35 ± 0.03^b	3.34 ± 0.06^b	3.35 ± 0.04^{bc}
96	3.32 ± 0.01^b	3.29 ± 0.01^b	3.30 ± 0.02^c
120	3.30 ± 0.01^b	3.30 ± 0.02^b	3.30 ± 0.01^c
144	3.34 ± 0.01^b	3.31 ± 0.01^b	3.32 ± 0.02^{bc}
168	3.31 ± 0.03^b	3.32 ± 0.01^b	3.31 ± 0.02^c
192	3.28 ± 0.01^b	3.35 ± 0.05^b	3.32 ± 0.05^c
216	3.32 ± 0.03^b	3.33 ± 0.02^b	3.33 ± 0.02^{bc}
240	3.37 ± 0.02^b	3.36 ± 0.03^b	3.36 ± 0.02^b
Mean across fermentation times	3.35 ± 0.09^a	3.34 ± 0.07^a	—

Note: Data were analyzed using a 2×10 factorial completely randomized design, with fermentation temperature (31 and 36 °C) and fermentation time (24–240 h) as the experimental factors. Mean differences were evaluated using Tukey's test at a significance level of 5% ($\alpha = 0.05$). Different superscript letters within the same column indicate statistically significant differences. Source: Research data, 2026.

3.1.2 TDS

The effects of fermentation temperature and duration on the total dissolved solids (TDS) of Arabica coffee fermentation liquid are presented in Table 2. The temperature $\times$ fermentation time interaction significantly affected TDS ($p < 0.01$). At 31 °C, TDS remained statistically stable throughout fermentation,

ranging from 2.41 ± 0.12 % at 24 h to 2.60 ± 0.17 % at 144 h, with all observation times belonging to the same significance group. In contrast, TDS at 36 °C exhibited an overall increasing trend, rising from 2.79 ± 0.19 % at 24 h to 3.26 ± 0.10 % at 240 h. The value recorded at 240 h was significantly higher than those at most other observation times, whereas the value at 144 h represented an intermediate response. Overall, prolonged fermentation increased TDS at 36 °C but did not produce a significant temporal change at 31 °C.

Table 2: Effects of fermentation temperature and time on the total dissolved solids (TDS) of Arabica coffee fermentation liquid.

Fermentation time (h)	31 °C	36 °C	Mean across temperatures
24	2.41 ± 0.12^a	2.79 ± 0.19^a	2.60 ± 0.25^b
48	2.60 ± 0.16^a	2.80 ± 0.22^a	2.70 ± 0.20^{ab}
72	2.56 ± 0.14^a	2.86 ± 0.04^a	2.71 ± 0.19^{ab}
96	2.57 ± 0.05^a	2.82 ± 0.07^a	2.70 ± 0.15^b
120	2.58 ± 0.14^a	2.87 ± 0.06^a	2.72 ± 0.19^{ab}
144	2.60 ± 0.17^a	2.98 ± 0.07^{ab}	2.79 ± 0.24^{ab}
168	2.52 ± 0.01^a	2.93 ± 0.03^a	2.72 ± 0.23^{ab}
192	2.50 ± 0.05^a	2.96 ± 0.03^a	2.73 ± 0.25^{ab}
216	2.52 ± 0.02^a	3.06 ± 0.03^a	2.79 ± 0.30^{ab}
240	2.54 ± 0.02^a	3.26 ± 0.10^b	2.90 ± 0.40^a
Mean across fermentation times	2.54 ± 0.10^b	2.93 ± 0.16^a	—

Note: Data were analyzed using a 2×10 factorial completely randomized design, with fermentation temperature (31 and 36 °C) and fermentation time (24–240 h) as the experimental factors. Mean differences were evaluated using Tukey's test at a significance level of 5% ($\alpha = 0.05$). Different superscript letters within the same column indicate statistically significant differences. Source: Research data, 2026.

3.1.3 °Brix (TSS)

The interaction between fermentation temperature and fermentation time did not significantly affect °Brix ($p > 0.05$), whereas the main effects of temperature and fermentation time were significant ($p < 0.05$). The effects of fermentation temperature and duration on the total soluble solids (TSS, °Brix) of Arabica coffee fermentation liquid are presented in Table 3. Fermentation temperature and duration significantly affected the °Brix value ($p < 0.05$). Across all fermentation times, the mean °Brix value at 36 °C was significantly higher than that at 31 °C, reaching 3.66 ± 0.20 and 3.16 ± 0.23 , respectively. Across both temperatures, the mean °Brix value increased from 3.08 ± 0.56 at 24 h to 3.68 ± 0.42 at 240 h. The intermediate observation times were assigned to overlapping statistical groups, indicating a gradual increase in soluble solids during fermentation. Overall, higher fermentation temperature and prolonged fermentation were associated with increased °Brix values.

Table 3: Changes in total soluble solids (°Brix) during fermentation at 31 and 36 °C.

Fermentation time (h)	31 °C	36 °C	Mean fermentation time
24	2.69 ± 0.43	3.49 ± 0.23	3.08 ± 0.56 ^a
48	3.25 ± 0.20	3.50 ± 0.26	3.38 ± 0.25 ^{ab}
72	3.20 ± 0.18	3.57 ± 0.05	3.38 ± 0.23 ^{abc}
96	3.22 ± 0.06	3.53 ± 0.09	3.37 ± 0.18 ^{ab}
120	3.22 ± 0.17	3.58 ± 0.08	3.40 ± 0.23 ^{bc}
144	3.24 ± 0.21	3.72 ± 0.03	3.48 ± 0.30 ^{bc}
168	3.16 ± 0.05	3.65 ± 0.03	3.40 ± 0.27 ^{bc}
192	3.10 ± 0.10	3.69 ± 0.02	3.39 ± 0.33 ^{abc}
216	3.27 ± 0.10	3.82 ± 0.02	3.54 ± 0.31 ^{bc}
240	3.31 ± 0.10	4.06 ± 0.06	3.68 ± 0.42 ^c
Temperature mean	3.16 ± 0.23 ^a	3.66 ± 0.20 ^b	

Note: Data are presented as mean ± standard deviation. Based on the analysis of variance, the interaction between fermentation temperature and fermentation time was not significant; therefore, superscript letters are shown only for the main-effect means. Different superscript letters within the same comparison group indicate significant differences among treatments at $p < 0.05$, corresponding to a 95% confidence level. Source: Research data, 2026.

3.2 Culturable bacterial succession during ohmic-assisted carbonic maceration

The dynamics of the dominant culturable bacterial genera and Gram-negative and Gram-positive bacterial counts during ohmic-assisted carbonic maceration of Arabica coffee are presented in Table 4. At 31 °C, Gram-negative bacteria predominated throughout the 24–240 h fermentation period, with counts ranging from 3.0×10^3 to 1.32×10^5 CFU/mL. Gram-positive bacteria were not detected at most observation times, except at 48 h, when their count reached 3.0×10^3 CFU/mL. The dominant culturable genera shifted from *Klebsiella* sp. at 24–96 h to *Acinetobacter* sp. at 120 h, *Pseudomonas* sp. at 144–168 h, and *Alcaligenes* sp. at 192–240 h.

At 36 °C, Gram-positive bacteria contributed more strongly during early to mid-fermentation, with counts of 1.0×10^3 CFU/mL at 24 h, 6.0×10^3 CFU/mL at 48 h, 2.0×10^3 CFU/mL at 72 h, and 6.3×10^4 CFU/mL at 96 h. Gram-positive bacteria were detected again at 192 h at 1.5×10^4 CFU/mL. Gram-negative counts ranged from 3.0×10^3 to 1.12×10^5 CFU/mL and increased during the late fermentation stage. The dominant culturable genera shifted from *Lactobacillus* sp. at 24–48 h to *Bacillus* sp. at 72–120 h, *Klebsiella* sp. at 144–192 h, and *Alcaligenes* sp. at 216–240 h. Overall, fermentation at 31 °C was characterized by persistent Gram-negative dominance, whereas fermentation at 36 °C exhibited a more pronounced contribution of Gram-positive bacteria during the early and middle stages.

3.3 Identification of Selected Bacterial Isolates Using VITEK MS MALDI-TOF MS

At 36 °C, selected isolates obtained at 72 h were identified using VITEK MS. The analysis identified the selected isolates as *Bacillus subtilis*, *B. amyloliquefaciens*, *B. vallismortis*, and *B. idriensis* at a confidence level of $\geq 99.9\%$, supporting the genus-level identification of the dominant culturable isolates during this phase.

4 DISCUSSION

4.1 Physicochemical dynamics during ohmic-assisted carbonic maceration

The fermentation system used in this study differed from classical carbonic maceration because external CO₂ was not injected into the fermenter at the beginning of the process. Instead, the closed vessel was designed to develop anaerobic conditions progressively through oxygen consumption and endogenous CO₂ production by the coffee cherries and microorganisms. An audible release of pressure was observed when the fermenter was opened at the end of fermentation, indicating the accumulation of fermentation-derived gas within the sealed vessel. This observation was consistent with probable CO₂ accumulation; however, it did not confirm the establishment of a CO₂-rich atmosphere because neither gas composition nor headspace pressure was measured directly. Accordingly, the process is interpreted as carbonic-maceration-based closed fermentation with self-induced anaerobiosis under ohmic temperature control (Brioschi Junior et al., 2021; Pereira et al., 2022; Da Silva Vale et al., 2023).

As shown in Table 3.1, a sharp decrease in pH was observed during 24–48 h at both temperatures, indicating rapid net acidification during the early fermentation stage. This pattern may reflect the conversion of fermentable substrates into organic acids by the active microbiota. The subsequent limited change in pH should not necessarily be interpreted as metabolic equilibrium; rather, it may reflect a balance among acid production, acid consumption, buffering by the coffee matrix, and declining availability of readily fermentable substrates. Because individual organic acids were not measured, their specific contributions to the observed pH trajectory cannot be determined. Nevertheless, pH remains a useful process indicator for monitoring the progression of coffee fermentation (Avallone et al., 2001; Haile & Kang, 2019; Tirado-Kulieva et al., 2024).

In closed coffee fermentation, temperature and duration can influence substrate conversion, microbial succession, and the accumulation or depletion of soluble compounds. The higher TDS and °Brix values observed at 36 °C therefore indicate a different physicochemical trajectory

Table 4: Dynamics of dominant bacterial genera and Gram-negative and Gram-positive bacterial counts (CFU/mL) during ohmic-assisted carbonic maceration at 31 and 36 °C for 24–240 h.

Temperature (°C)	Time (h)	Genus	Gram-negative (CFU/mL)	Gram-positive (CFU/mL)
31	24	<i>Klebsiella</i> sp.	5.4×10^4	0
	48	<i>Klebsiella</i> sp.	3.0×10^3	3.0×10^3
	72	<i>Klebsiella</i> sp.	7.0×10^4	0
	96	<i>Klebsiella</i> sp.	1.32×10^5	0
	120	<i>Acinetobacter</i> sp.	3.5×10^4	0
	144	<i>Pseudomonas</i> sp.	1.05×10^5	0
	168	<i>Pseudomonas</i> sp.	3.0×10^4	0
	192	<i>Alcaligenes</i> sp.	1.2×10^4	0
	216	<i>Alcaligenes</i> sp.	1.4×10^4	0
	240	<i>Alcaligenes</i> sp.	6.0×10^4	0
36	24	<i>Lactobacillus</i> sp.	3.0×10^3	1.0×10^3
	48	<i>Lactobacillus</i> sp.	6.0×10^3	6.0×10^3
	72	<i>Bacillus</i> sp.	1.3×10^4	2.0×10^3
	96	<i>Bacillus</i> sp.	2.7×10^4	6.3×10^4
	120	<i>Bacillus</i> sp.	2.9×10^4	2.7×10^4
	144	<i>Klebsiella</i> sp.	1.8×10^4	0
	168	<i>Klebsiella</i> sp.	1.0×10^4	0
	192	<i>Klebsiella</i> sp.	1.3×10^4	1.5×10^4
	216	<i>Alcaligenes</i> sp.	6.0×10^4	0
	240	<i>Alcaligenes</i> sp.	1.12×10^5	0

Note: The dominant culturable genus at each observation time was determined based on the colony type occurring most frequently on Plate Count Agar. Gram-negative and Gram-positive bacterial counts were assigned according to the Gram-staining characteristics of representative isolates recovered from the counted plates. Fermentation samples were collected independently from three fermentors for each temperature treatment. Where replicate count data from the three independent fermentors were available, culturable bacterial counts are presented as mean $\pm$ standard deviation. Where only a single representative count or pooled morphological classification was available, the values are presented descriptively and were not treated as replicate means. Values are expressed as colony-forming units per millilitre (CFU/mL). A value of 0 indicates that no colony belonging to the corresponding Gram group was detected under the cultivation and incubation conditions used. The results represent only the culturable bacterial population and do not provide a comprehensive profile of the entire microbial community.

Source: Research data, 2026.

from that observed at 31 °C. However, these refractometric measurements represent composite responses to all dissolved constituents and cannot distinguish among sugars, organic acids, minerals, aroma precursors, and other metabolites. Consequently, the observed increases should be interpreted as changes in the overall soluble fraction rather than as direct measurements of sugar production or consumption or as evidence of enhanced sweetness, body, or aroma complexity. Complementary analyses of individual sugars, organic acids, volatile compounds, and sensory attributes would be required to determine the compounds responsible for these changes and their potential quality implications (Osorio Pérez et al., 2022; Osorio et al., 2023; Ferreira et al., 2023; Tirado-Kulieva et al., 2024; Dos Santos Gomes et al., 2025).

The more pronounced increases in TDS and °Brix at 36 °C may have resulted from enhanced mass transfer and

solubilisation of mucilage and pulp constituents, together with changes in sugars, organic acids, and other fermentation products. Nevertheless, the late increase cannot be attributed exclusively to microbial metabolism. Repeated liquid removal, changes in headspace volume, possible water loss, and concentration effects may also have influenced the values. A mass balance and direct analysis of sugars and organic acids would therefore be required to determine whether the increase resulted mainly from biochemical transformation, solute release, or physical concentration of the fermentation liquid (Osorio Pérez et al., 2022; Osorio et al., 2023; Peñuela-Martínez et al., 2025).

Ohmic heating enabled the two fermentation-temperature set points to be established through volumetric heat generation and may have reduced temperature gradients within the vessel. However, because no conventional-heating control was operated

at the same temperatures, the present design cannot distinguish the effects of temperature from any additional thermal or non-thermal effects of the electric field. The findings should therefore be described as temperature effects observed within an ohmic-controlled system, rather than as evidence that ohmic heating itself accelerated microbial activity or metabolite formation. Reporting the actual temperature profile, electrical conductivity, current, voltage, and energy input would further strengthen this interpretation (Gavahian & Tiwari, 2020; Alkanan et al., 2021; Javed et al., 2025).

The pH, TDS, and °Brix trajectories provide indirect information regarding the intensity and progression of biochemical transformations. In a related study conducted using the same fermentation system, Mustafa et al. (2026) reported temperature and time dependent changes in chlorogenic acids, alkaloids, sugars, volatile compounds, and cup quality at 31 and 36 °C over fermentation periods of 48, 144, and 240 h. These findings demonstrate the broader relevance of temperature and fermentation duration to chemical and sensory changes. However, they do not establish a direct causal relationship between the TDS and °Brix trajectories observed in the present study and specific chemical or sensory outcomes. Because individual sugars, organic acids, volatile compounds, and sensory attributes were not measured in the present study, the observed physicochemical patterns should be interpreted as process indicators with potential quality relevance rather than as direct evidence of sensory improvement (Brioschi Junior et al., 2021; Gomes et al., 2022; Mustafa et al., 2026; Dos Santos Gomes et al., 2025).

Prolonged fermentation at warmer temperatures may increase the risk of undesirable sensory changes. Previous carbonic-maceration studies have shown that fermentation temperature and duration can alter volatile and sensory profiles, thereby affecting the balance between desirable fruity or complex attributes and potentially over-fermented, excessively sour, vinegary, or solvent-like characteristics. However, these sensory risks cannot be confirmed from the present study because volatile compounds and sensory attributes were not directly evaluated (Dos Santos Gomes et al., 2025).

4.2 Temperature-dependent culturable bacterial succession

Descriptively, the culturable bacterial profile at 36 °C showed a greater contribution of Gram-positive isolates during early to mid-fermentation than that observed at 31 °C. This pattern suggests a temperature-associated difference in the organisms recovered under the applied culture conditions. However, because the microbial counts were not subjected to inferential statistical analysis and were obtained using a single non-selective medium, the results should not be interpreted

as definitive evidence that temperature caused the observed community shift. Culture conditions, storage before analysis, and colony-selection procedures may also have influenced which organisms were recovered (Silva et al., 2008; De Bruyn et al., 2017; Pereira et al., 2022).

The identification of several *Bacillus* species at 72 h, together with the higher Gram-positive count recorded at 96 h, indicates that the 72–96 h period may represent a candidate transition phase in the culturable bacterial profile at 36 °C. This interpretation must remain cautious because MALDI-TOF MS identification was conducted only for selected isolates obtained at 72 h and did not confirm the species present at 96 h or at other temperatures. In addition, enzyme activities and metabolic products associated with these isolates were not measured. The functional contribution of *Bacillus* to substrate conversion therefore requires direct validation rather than inference from taxonomic identification alone (Da Silva et al., 2023; Cortés et al., 2024).

The recovery of Gram-negative and facultative bacteria during the early phase at 31 °C is consistent with the heterogeneous epiphytic microbiota associated with coffee cherries and with the presence of residual oxygen at the beginning of closed fermentation. At 36 °C, the recovery of *Lactobacillus* and *Bacillus* during early to mid-fermentation may indicate selection associated with temperature, acidification, substrate availability, and the progressively changing atmosphere. However, because only culturable bacteria on PCA were investigated, these results do not represent the full succession of LAB, acetic acid bacteria, yeasts, or filamentous fungi involved in coffee fermentation (Avallone et al., 2001; Silva et al., 2008; De Carvalho Neto et al., 2017; Haile & Kang, 2019).

The recovery of *Pseudomonas* spp. and *Alcaligenes* sp. during the middle and late fermentation stages appears unexpected in a system intended to become progressively anaerobic. Their occurrence may reflect oxygenated microniches, air ingress during sampling, the progressive increase in headspace volume caused by daily liquid removal, or preferential recovery of rapidly growing aerobic organisms on PCA. However, these explanations remain hypothetical because dissolved oxygen and headspace O₂ and CO₂ were not measured. Freezing and thawing before enumeration and the selection of representative colony morphotypes may also have influenced the bacterial profile recovered under the applied cultivation conditions (Pereira et al., 2022; Da Silva Vale et al., 2023; Peñuela-Martínez et al., 2025).

4.3 Relationships between culturable bacterial succession and physicochemical trajectories

The rapid pH decrease observed between 24 and 48 h occurred concurrently with the early recovery of

Lactobacillus and other culturable bacteria at 36 °C, whereas the later increases in TDS and °Brix coincided with subsequent changes in the dominant culturable genera. These parallel patterns suggest that changes in the fermentation environment and culturable bacterial profile occurred over similar time scales. However, no formal correlation, regression, or multivariate analysis was conducted, and the microbiological observations were culture dependent. The results therefore indicate temporal association rather than a demonstrated causal relationship among bacterial succession, acidification, and dissolved-solids dynamics (Tirado-Kulieva et al., 2024; Todhanakasem et al., 2024).

These temporal patterns are consistent with the broader chemical and sensory changes reported in a related study conducted using the same fermentation system; however, the microbial, physicochemical, chemical, volatile, and sensory datasets were not statistically integrated. The findings should therefore be interpreted as parallel process patterns rather than as evidence that particular culturable bacterial genera directly caused the reported chemical or sensory changes (Mustafa et al., 2026).

4.4 Methodological limitations

This study had methodological limitations that must be emphasised. Microbiological enumeration was conducted using PCA as a non-selective medium; therefore, the results primarily represent the culturable bacterial load under the incubation conditions used rather than a comprehensive microbial-community profile. Identification was based on dominant colonies and did not include yeasts or filamentous fungi. Future studies should employ multiple selective media and/or culture-independent methods, particularly for closed or SIAF fermentations (Da Silva Vale et al., 2023; Pereira et al., 2022).

5 CONCLUSIONS

Controlled ohmic fermentation at 31 and 36 °C produced distinct physicochemical and culturable bacterial trajectories in whole-cherry Arabica coffee. Both temperatures caused rapid acidification within 24–48 h, whereas TDS and °Brix increased more markedly at 36 °C. Fermentation at 31 °C remained predominantly Gram-negative, while 36 °C promoted a mixed community with a critical *Bacillus*-associated transition at 72–96 h. These findings support precise temperature and headspace control for more reproducible carbonic/SIAF fermentation.

6 AUTHOR CONTRIBUTIONS

Conceptual idea: Mustafa, A.; Methodology design: Salengke, S., and Bastian, F.; Data collection: Mustafa, A.; Data analysis and interpretation: Dirpan, A., Mursalim, and Laga, A.; Writing and editing: Laga, A., Hasizah, A., and Ilham, A.

7 ACKNOWLEDGMENTS

The authors acknowledge the support from the Ministry of Education and Research for funding this research through the Doctoral Dissertation Research Scheme with the contract number 124/E5/PG.02.00.PL/2023.

8 DATA AVAILABILITY STATEMENT

Data available upon request to authors.

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