

Preliminary Data on Monitoring the Fermentation of Liberica Coffee using a Sensor-Integrated Mini Bioreactor

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ABSTRACT

Fermentation in bioreactor systems can enhance coffee quality, standardize microbial activity, and improve the reproducibility of specialty coffee production. This study evaluated the performance of a custom mini-bioreactor with real-time gas sensors for monitoring the fermentation of *Coffea liberica* cherries inoculated with *Bacillus cereus*. The system continuously measured CO₂, H₂, CH₄, ethanol, temperature, and pH, enabling a comprehensive profiling of fermentation. Sensory evaluation and LC-MS chemical fingerprinting assessed the impact of microbial fermentation on coffee quality. Fermentation with *B. cereus* (FCB) produced higher levels of CO₂ (545 ppm), H₂ (379.11 ppm), ethanol (472.32 ppm), and CH₄ (1.85 ppm) than fermentations with *Leuconostoc* (FBL) and *Exiguobacterium indicum* (FBE), except for CH₄. LC-MS analysis confirmed that fermentation preserved the core chemical profile of Liberica coffee, with caffeine as the dominant compound. These results show that sensor-integrated bioreactor systems offer a scalable, controllable platform for real-time monitoring and optimization of coffee fermentation.

Key words: *Bacillus cereus*; coffee fermentation; mini-bioreactor; gas-exhaust.

1 INTRODUCTION

Fermenting Liberica coffee (*Coffea liberica*) has shown promising potential as a post-harvest method to improve its sensory quality, chemical profile, and marketability. Unlike *Coffea arabica* and *Coffea canephora* (Robusta), which together account for over 90% of global coffee production and consumption (International Coffee Organization, ICO, 2022), Liberica remains underused despite its unique flavor, resilience to climate change, and disease resistance (Banti & Atlaw, 2024; Cheserek et al., 2021). Targeted microbial fermentation can help standardize its flavor and reduce post-harvest variability, making Liberica more competitive in the specialty coffee market (De Bruyn et al., 2017; Elhalis et al., 2020; Tolentino Vaz et al., 2022).

Recent studies have demonstrated that controlled fermentation using selected microbial strains can modulate biochemical pathways that create key aroma and flavor precursors in coffee beans (Pereira et al., 2017; Salengke et al., 2019). In particular, fermentation with *Bacillus spp.* has increased the production of volatile compounds and improved cup quality of Liberica coffee (Latief et al., 2023; Somporn et al., 2011; Tarigan et al., 2024a; Tarigan et al., 2024b). Biotechnological tools such as bioreactors and real-time sensors offer new ways to optimize Liberica fermentation and increase its global market value.

In vitro fermentation has been shown to improve both the sensory profiles and the bioactive compound content of coffee

(De Bruyn et al., 2017), while roasting further shapes flavor characteristics and contributes to the beverage's environmental footprints (Toci & Farah, 2008). During coffee fermentation, microbes break down complex macromolecules into simpler compounds, leading to major chemical transformations in the beans (Towaha & Rubiyo, 2016). Natural fermentations, such as those occurring in civet coffee, can reduce caffeine levels and modify flavor by increasing fatty acids like palmitic, lauric, linoleic, and docosahexaenoic acids (Bressani et al., 2018). Enzymatic activity also decreases caffeine, chlorogenic acid, and proteins while increasing polysaccharide and lipid concentrations, leading to richer taste and enhanced aroma (De Carvalho Neto et al., 2018). Although civet coffee is highly valued for its unique flavor and origin, it faces limitations related to high operational costs and sustainability concerns, particularly animal welfare (Jumhawan et al., 2013, 2015; Putri et al., 2015). To overcome these challenges and ensure consistent coffee quality, bioreactor-based systems offer a promising alternative. Bioreactors provide controlled fermentation with real-time monitoring of critical parameters, improving process stability and providing insights into the biochemical changes occurring during fermentation.

A bioreactor is a closed system designed to optimize biological processes under controlled conditions including temperature, pH, oxygen availability, and agitation (Martinez et al. 2021). In coffee fermentation, bioreactors provide a reproducible and sterile environment that supports consistent microbial activity and metabolite production. Systems

incorporating sequential aerobic and anaerobic phases have shown promise in promoting starter culture growth, enhancing sugar metabolism, and generating flavor-related compounds (Elhalis et al., 2023; Martinez et al., 2021)

By maintaining optimal biochemical conditions, bioreactors improve fermentation yield and provide a robust platform for monitoring complex metabolic pathways (Reyes et al., 2024). When integrated with real-time gas sensors, they enable dynamic tracking of key indicators (e.g., CO₂, ethanol, and redox shifts) critical for flavor control and process consistency. Martinez et al. (2021) reported that bioreactors equipped with impellers improve metabolite distribution and microbial contact in the coffee mass. Several studies highlight bioreactors' potential in specialty coffee innovation. For instance, Hadipernata and Nugraha (2018) successfully produced civet-like coffee through microbial fermentation in a bioreactor, achieving comparable sensory profiles. Anaerobic bioreactors also support biogas production (e.g., H₂ and CH₄), offering energy and environmental benefits (Shah et al., 2024; Sridevi et al., 2024). In addition, these systems generate organic acids and volatiles like lactic acid, acetic acid, and ethanol that strongly influence the final cup profile (Galarza & Figueroa, 2022). Despite these advantages, the high cost of stainless-steel bioreactors remains a barrier to widespread use. Fermentation in such systems can be considerably more expensive than in traditional tanks, pointing to the need for affordable and scalable bioreactor models tailored to the coffee sector (Magalhães Júnior et al., 2021). Here, we evaluated a custom mini bioreactor integrated with gas sensors to monitor the fermentation of *Coffea liberica* cherries inoculated with *Bacillus cereus*.

2 MATERIAL AND METHODS

2.1 Chemicals and equipments

Liberica coffee cherries (Grade B) were collected from Betara District, Jambi Province, Indonesia. The coffee beans (*Coffea liberica*) originated from a certified local plantation in the same region. Bacterial inoculants—cellulolytic strains of *Bacillus subtilis*, *Leuconostoc* spp., and *Exiguobacterium indicum*—were previously isolated, identified, and maintained in the culture collection of the Natural Product and Bioactive Compound Laboratory. The strains were obtained from the Indonesian Agency for Research and Innovation (BRIN) under the IACC collection.

Bacteria were cultivated in de Man, Rogosa, and Sharpe (MRS) broth (Merck, Germany) and on nutrient agar (NA) (Merck, Germany). Additional analytical-grade reagents included sterile cotton swabs, distilled water (aquadest), phosphate buffer, 70% ethanol, 80% methanol, 70% methanol, and sodium hydroxide (NaOH), all purchased from Sigma-Aldrich (USA).

The equipment used in this study comprised an analytical balance (Ohaus Pioneer PA214), a hot plate stirrer (IKA C-MAG HS 7), a small-batch coffee roaster (Probenroaster PRE-IZ, Germany), and a laboratory-grade grinder (Rancilio Rocky SD). Microbiological and analytical instruments included inoculating loops, separatory funnels, evaporating dishes, Petri dishes, a pH meter (Hanna Instruments HI2211), a desiccator, standard chemical glassware (beakers, flasks, and measuring cylinders), a magnetic stirrer, and a thermostatic water bath (Mettmert WNB Series).

2.2 Design and description of the stainless steel mini bioreactor

A custom-built stainless-steel mini bioreactor equipped with an integrated monitoring and control system was developed for this study (Figure 1). The system was designed to regulate key fermentation parameters, including pH, temperature, and gas composition (CO₂, CH₄, H₂, and C₂H₅OH), using a set of high-sensitivity sensors (Table 1) strategically positioned between the inner and outer vessel lids. A DC motor, connected to a programmable delay system, was configured to activate every 30 minutes, driving three vertical impellers arranged in a multi-stirrer configuration. This setup ensured homogeneous mixing and maintained a uniform turbulent flow, which is critical for microbial fermentation and other biochemical processes requiring precise environmental control.

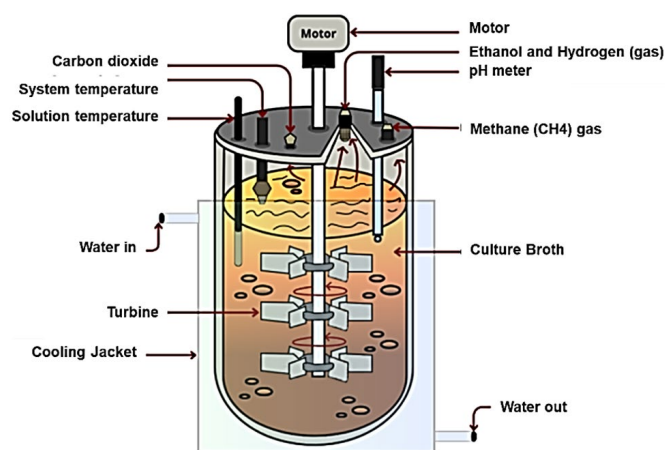


Figure 1: Design of the mini bioreactor with its sensor-integrated monitoring system.

The reactor was further equipped with a passive cooling jacket designed to circulate chilled water around the vessel, allowing temperature regulation without the need for active refrigeration. The system's operational data were processed through an Arduino Mega 2560 microcontroller, programmed in C++ using the Arduino Integrated Development

Environment (IDE). Real-time sensor data were continuously visualized and logged into Microsoft Excel 2010 using the Data Streamer extension, enabling synchronized monitoring and data acquisition throughout the fermentation process.

2.3 Inoculum preparation

fermentation experiments were conducted in a stainless-steel mini bioreactor using 1 kg of coffee material per batch, with all treatments performed in triplicate. Three bacterial inoculation treatments were applied: *Bacillus cereus* (FBC) on coffee cherries, *Leuconostoc* sp. (FGL) on green beans, and *Exiguobacterium indicum* (FGE) on green beans. Bacterial starter cultures were reactivated in de Man, Rogosa, and Sharpe (MRS) broth at 37 °C for 24 h prior to inoculation, then standardized to a cell density of approximately 10^7 – 10^8 CFU/mL, corresponding to an optical density (OD₆₀₀) of 0.8–1.0, which is within the range commonly used for controlled coffee and food fermentations (Lee et al., 2015; De Melo Pereira et al., 2019). A 5% (v/w) inoculum was introduced directly into the bioreactor and thoroughly mixed with the coffee substrate to ensure homogeneous cell distribution, resulting in an estimated initial microbial load of 10^6 – 10^7 CFU/g of fermentation mass. After fermentation, the processed coffee samples were dried to approximately 12% (w/w) moisture content to ensure post-fermentation stability and prevent microbial regrowth during storage (Tarigan et al., 2024a; Tarigan et al., 2024b).

2.4 Research flowchart

The initial stage of system development involved mechanical design, component selection, and preliminary testing to verify system functionality. The fabrication workflow of the mini bioreactor, including the monitoring and control stages, is illustrated in Figure 2. The process begins with device initiation, followed by sensor activation for gas and temperature detection. Once the sensors detect the environmental parameters, data are transmitted to the Arduino ATmega 2560 microcontroller for processing. The processed data are then displayed through the user interface for real-time

monitoring. If no signal is detected, the system automatically reinitializes to maintain continuous data acquisition.

2.5 Temperature and pH monitoring

Temperature in the mini bioreactor was controlled manually during fermentation by circulating water around the vessel. Heat transfer occurred through conduction and convection to maintain stable conditions. FCB samples were monitored to keep both temperature and pH stable. The pH was adjusted using NaOH to maintain neutral acidity (Hadipernata & Nugraha, 2018).

2.6 Gas monitoring

Gas concentration profiles were monitored throughout the fermentation process using a 4.2 L stainless-steel mini bioreactor equipped with integrated gas sensors. Three types of semiconductor gas sensors were employed: MQ-2 for hydrogen and ethanol vapor detection, MQ-4 for methane (CH₄), and MG811 for carbon dioxide (CO₂). These sensors were positioned within the headspace of the bioreactor to capture real-time changes in gaseous metabolites produced during microbial activity. Prior to fermentation, each sensor was calibrated and tested for sensitivity to ensure accuracy and stability of readings. The calibration procedure involved exposing the sensors to a controlled gas source generated from a butane flame igniter, allowing response verification under known conditions. Voltage outputs (V) were recorded both before and after gas exposure to determine baseline signal stability and response amplitude. The calibration data were used to define the operational response range and to normalize voltage readings during actual fermentation monitoring.

During the fermentation trials, the sensors continuously measured headspace gas composition, and voltage signals were logged by the Arduino Mega 2560 microcontroller at predetermined intervals. The recorded data were converted into relative concentration values based on the calibration curves of each sensor. The monitoring system thus enabled real-time observation of gas production dynamics, providing indirect insights into microbial metabolism and fermentation kinetics.

Table 1: Sensors integrated into the mini bioreactor and their functions.

Sensor	Measured parameter	Function / Description
DS18B20	Solution temperature	Monitors the temperature of the fermentation medium to maintain thermal stability.
pH Electrode E201-C	Solution pH	Measures the pH of the fermentation broth for process control and optimization.
Type-K Thermocouple Sensor with MAX6675	Ambient/fermentation temperature	Detects the ambient and internal fermentation temperature for comparative calibration.
MQ-2	Ethanol and hydrogen (H ₂) gases	Detects ethanol vapor and hydrogen gas concentration during fermentation.
MG811	Carbon dioxide (CO ₂) gas	Monitors CO ₂ evolution as an indicator of microbial metabolic activity.
MQ-4	Methane (CH ₄) gas	Detects methane generated from anaerobic microbial activity.
DFRobot pH Sensor SEN0161-V2	pH monitoring	Provides redundant pH control and data validation through dual-sensor configuration.

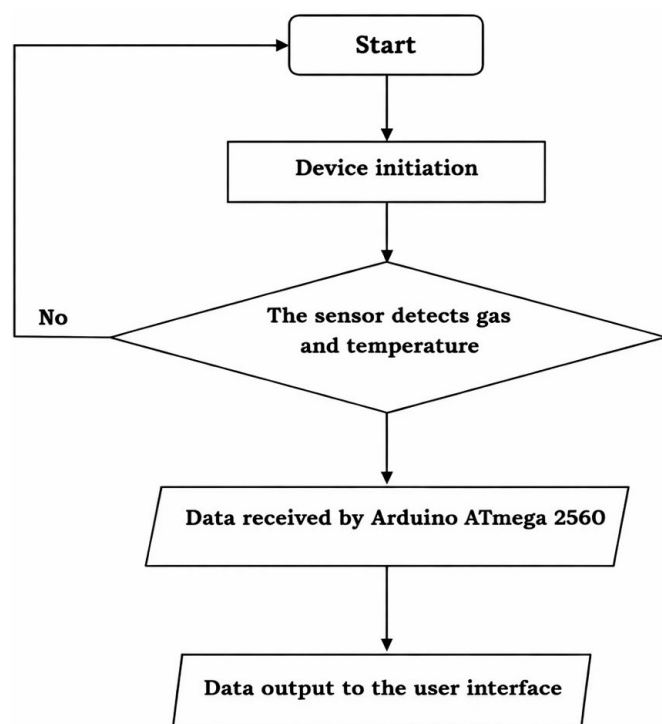


Figure 2: Flowchart of the monitoring process during fermentation.

2.7 Cup Test (Sensory Evaluation)

The sensory evaluation of roasted *Coffea liberica* cherries inoculated with *Bacillus cereus* (FCB) was performed following the official cupping protocol established by the Specialty Coffee Association of America (SCAA). This evaluation aimed to determine the sensory quality and flavor characteristics of the fermented coffee in comparison with established specialty coffee standards. The cupping procedure was conducted in a controlled sensory laboratory environment to minimize external bias. Coffee samples were roasted to a medium profile and ground immediately before testing. Each sample was brewed using freshly boiled water maintained between 92 and 96 °C, with a fixed coffee-to-water ratio of 8.25 g per 150 mL. The brewing process was carried out in standardized white porcelain cupping bowls to ensure uniform thermal conditions and prevent color-based bias during sensory perception.

Three certified Q-graders, trained and accredited under SCAA standards, independently assessed the sensory attributes of the samples. The evaluated parameters included fragrance/aroma, flavor, aftertaste, acidity, sweetness, mouthfeel, balance, uniformity, clean cup, and overall impression. Each attribute was scored on a 10-point SCAA scale, where values between 6.00 and 6.99 were categorized as Good, 7.00–7.74 as Very Good, 7.75–8.74 as Excellent, and 8.75–10.00 as Outstanding. During the evaluation, panelists recorded both quantitative scores and qualitative sensory

notes. The fermented coffee exhibited a distinctive and pleasant fragrance characterized by floral and nutty aromatic notes, complemented by mild cocoa and caramel undertones in flavor. The aftertaste was clean and persistent, with a balanced acidity described as bright but not overpowering. The sweetness was natural and well-integrated with a smooth mouthfeel, providing a rounded and harmonious cup profile. All evaluations were conducted in triplicate to ensure data reliability, and the average scores were calculated from the independent assessments of the three Q-graders. The results suggest that microbial fermentation with *B. cereus* may contribute positively to the development of desirable sensory attributes, potentially improving the overall cup quality of *Coffea liberica* beans (Tarigan et al., 2024a).

2.8 Metabolite analysis by LC-MS

The metabolite profiling of *Coffea liberica* extracts was carried out using Ultra-Performance Liquid Chromatography–Mass Spectrometry (UPLC–MS) at the Bogor Forensic Laboratory Center, Indonesia. Only roasted coffee cherries inoculated with *Bacillus cereus* (FCB) were analyzed. Roasted samples were macerated in 97% (v/v) ethanol for three days at room temperature, followed by filtration and concentration of the filtrates using a thermostatic water bath until a semi-solid extract was obtained. The concentrated extracts were filtered through a 0.22 µm syringe filter prior to injection into the UPLC–MS system. Chromatographic separation was performed using an ACQUITY UPLC® H-Class system (Waters Corporation, Milford, MA, USA) equipped with an ACQUITY UPLC® HSS C18 column (1.8 µm, 2.1 × 100 mm). The column temperature was maintained at 50 °C, while the ambient temperature was 25 °C. The mobile phase consisted of (A) water containing 5 mM ammonium formate and (B) acetonitrile containing 0.05% formic acid. The elution was carried out using a gradient flow at a rate of 0.20 mL/min for a total run time of 23 min, with an injection volume of 5 µL. Mass spectrometric detection was performed using a Xevo G2-S QToF mass spectrometer (Waters, USA) operated in positive electrospray ionization (ESI⁺) mode. The analysis range was set at m/z 50–1200, with a source temperature of 100 °C, desolvation temperature of 350 °C, and desolvation gas flow of 793 L/h. The collision energy was maintained at 4 V (low energy) and 25–60 V (high energy). Chromatographic and spectral data were acquired and processed using MassLynx V4.1 software (Waters Corporation). Tentative identification of metabolites was achieved by comparing MS and MS/MS fragmentation spectra with reference data from established online databases, including ChemSpider, PubChem, MassBank, and the Human Metabolome Database (HMDB). Compound identities were assigned based on accurate mass measurements, isotope patterns, and fragmentation profiles.

3 RESULTS

3.1 Validation of sensors in the mini bioreactor

The sensor integration and validation process were conducted to ensure the accuracy, reliability, and real-time functionality of the monitoring system developed for the mini bioreactor. The overall system configuration is illustrated in the block diagram (Figure 3), which outlines the primary components, core operational processes, and their interconnections. The system continuously acquired data from multiple sensors responsible for monitoring key fermentation parameters, including gas composition (CO_2 , CH_4 , H_2 , and $\text{C}_2\text{H}_5\text{OH}$), pH, and temperature. These sensors were strategically positioned within the bioreactor to capture representative environmental conditions during the fermentation process. The sensor outputs were transmitted to an Arduino Mega 2560 microcontroller, which served as the central data acquisition unit.

The microcontroller was programmed to collect and process analog voltage signals from the sensors, converting them into digital data for subsequent transfer to a connected computer interface. The processed data were visualized in real time and recorded in Microsoft Excel via the Data Streamer add-in, facilitating synchronized data logging for further analysis.

To maintain a homogeneous fermentation environment, a DC motor-driven stirrer was integrated into the system. The motor operated under a controlled rotational cycle to ensure consistent agitation, which prevented sedimentation and ensured even distribution of microbial cells and nutrients. This configuration minimized environmental gradients and improved the precision of sensor readings across the entire fermentation vessel.

Sensor validation involved verifying the stability and responsiveness of each sensor under controlled experimental

conditions. Calibration curves were established for gas sensors, temperature probes, and the pH electrode prior to operation. Repeated measurements were performed to evaluate reproducibility and eliminate signal drift over time. The system's performance demonstrated a high degree of synchronization between data acquisition and environmental control, confirming its suitability for continuous fermentation monitoring.

3.2 pH and Temperature Changes During Fermentation

Monitoring pH and temperature throughout the fermentation process provides essential information regarding microbial activity, metabolic dynamics, and process stability, all of which directly affect the biochemical composition and overall quality of the coffee (Figures 4 and 5). The pH profile of the FCB (fermented coffee cherries inoculated with *Bacillus cereus*) exhibited a series of periodic and pronounced decreases during the early and mid-stages of fermentation (Figure 4A). These fluctuations reflect intensive microbial activity associated with the production of organic acids, primarily lactic, acetic, and other short-chain fatty acids generated by fermentative bacteria. The transient recovery of pH observed during the later stages suggests a shift in metabolic balance, possibly due to the consumption or neutralization of organic acids as microbial growth stabilized.

In contrast, the pH profiles of the FGE (fermented green coffee with *Exiguobacterium indicum*) and FGL (fermented green coffee with *Leuconostoc* spp.) treatments remained comparatively stable over the entire fermentation period (Figure 4B). The minimal pH variation in these treatments indicates lower acidogenic activity and a more controlled microbial metabolism, which may be attributed to the different enzymatic capabilities and metabolic pathways of the inoculated bacterial strains.

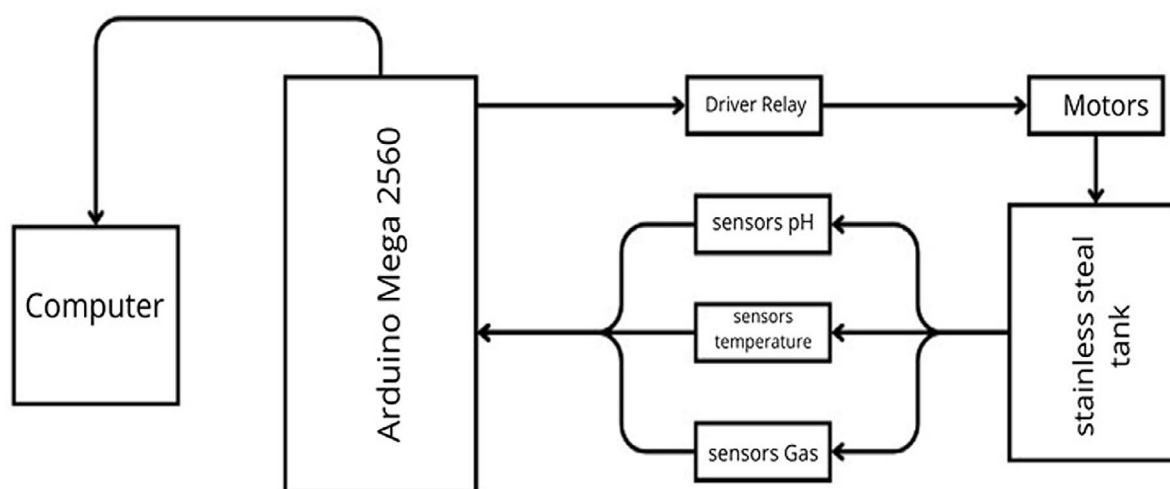


Figure 3: Block diagram of the mini-bioreactor with integrated sensors and control system.

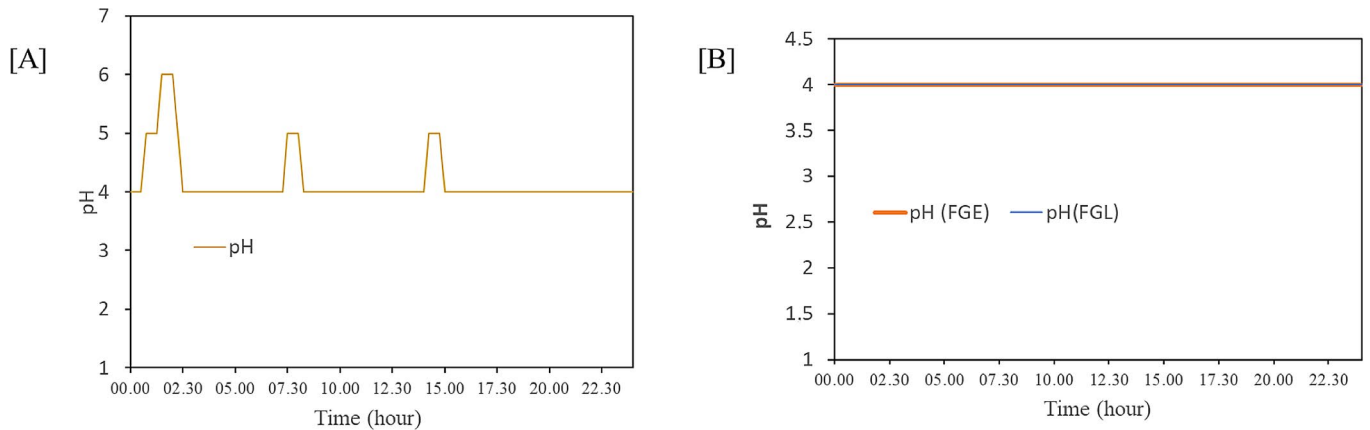


Figure 4: pH changes during fermentation. (A) FCB, (B) FGE and FGL.

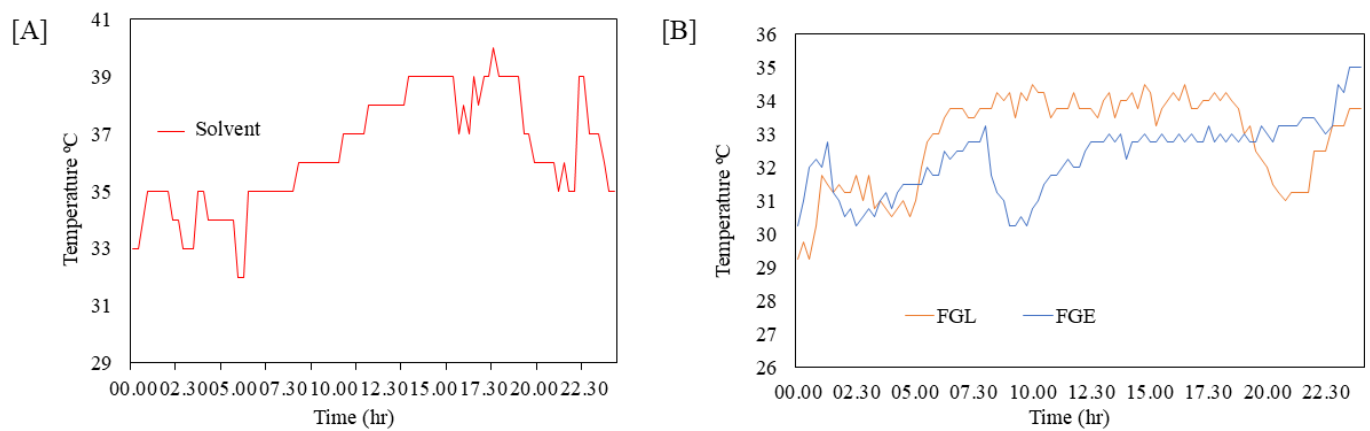


Figure 5: Temperature changes during fermentation in different samples (A) FCB, (B) FGL, and FGE.

Temperature monitoring revealed a gradual increase in the bioreactor's internal temperature during the first phase of fermentation, corresponding to elevated microbial metabolic rates and exothermic biochemical reactions (Figure 5). The temperature then reached a steady state as the fermentation progressed, suggesting a balance between microbial heat generation and the cooling capacity of the system's water-jacket mechanism. Collectively, the observed changes in pH and temperature demonstrate distinct microbial and physicochemical behaviors across different inoculation treatments. These findings highlight the influence of microbial strain selection on fermentation kinetics, organic acid formation, and thermal dynamics within the bioreactor, which ultimately determine the development of the coffee's sensory and chemical characteristics.

The temperature of the FCB solution (Figure 5A) fluctuated with peaks around 33–37°C, while the FGE and FGL solutions (Figure 5B) showed a gradual increase. In average, the FGL solution consistently reached higher temperatures than FGE, possibly due to more intense microbial activity or greater substrate availability.

3.3 Gas-exhaust monitoring during fermentation

Gas-emission profiles were continuously monitored throughout the fermentation process to evaluate microbial metabolic dynamics under different bacterial inoculations (Figure 6). In the initial phase of fermentation (0–1.5 h), gas concentrations across all treatments remained low, corresponding to the microbial adaptation or lag phase, during which cells adjust to environmental and nutritional conditions prior to exponential growth.

In the FCB treatment (coffee fermented with *Bacillus cereus*), data obtained from the MQ-2 sensor revealed a progressive increase in hydrogen (H_2) and ethanol (C_2H_5OH) concentrations following the lag phase, reaching maximum values at approximately 17 hr post-inoculation (Figure 6A). This increase indicates active fermentative metabolism, characterized by carbohydrate catabolism and alcohol production typically associated with facultative anaerobic processes. The subsequent decline in gas production after the peak suggests substrate depletion or a metabolic shift toward secondary pathways. In contrast, both the FGL (fermentation with *Leuconostoc* spp.) and FGE (fermentation with

Exiguobacterium indicum) treatments exhibited negligible H_2 production throughout the fermentation period. This result reflects the distinct metabolic characteristics of these strains, which are known to favor lactic and heterofermentative acid pathways over hydrogenogenic metabolism. Carbon dioxide (CO_2) production, monitored using the MG811 sensor, exhibited treatment-specific patterns (Figures 6C and 6D). Under ambient conditions, the baseline atmospheric CO_2 concentration was approximately 399 ppm, consistent with reference values reported by the Meteorology, Climatology,

and Geophysics Agency (BMKG, Indonesia). During fermentation, CO_2 levels remained relatively constant at baseline values in the FGL and FGE treatments, indicating limited respiratory or fermentative gas output. Conversely, the FCB treatment showed a steady increase in CO_2 concentration, peaking at approximately 590 ppm, which corresponded with the period of heightened H_2 and ethanol evolution. This co-occurrence strongly suggests active decarboxylation reactions and enhanced microbial respiration during the mid-fermentation phase.

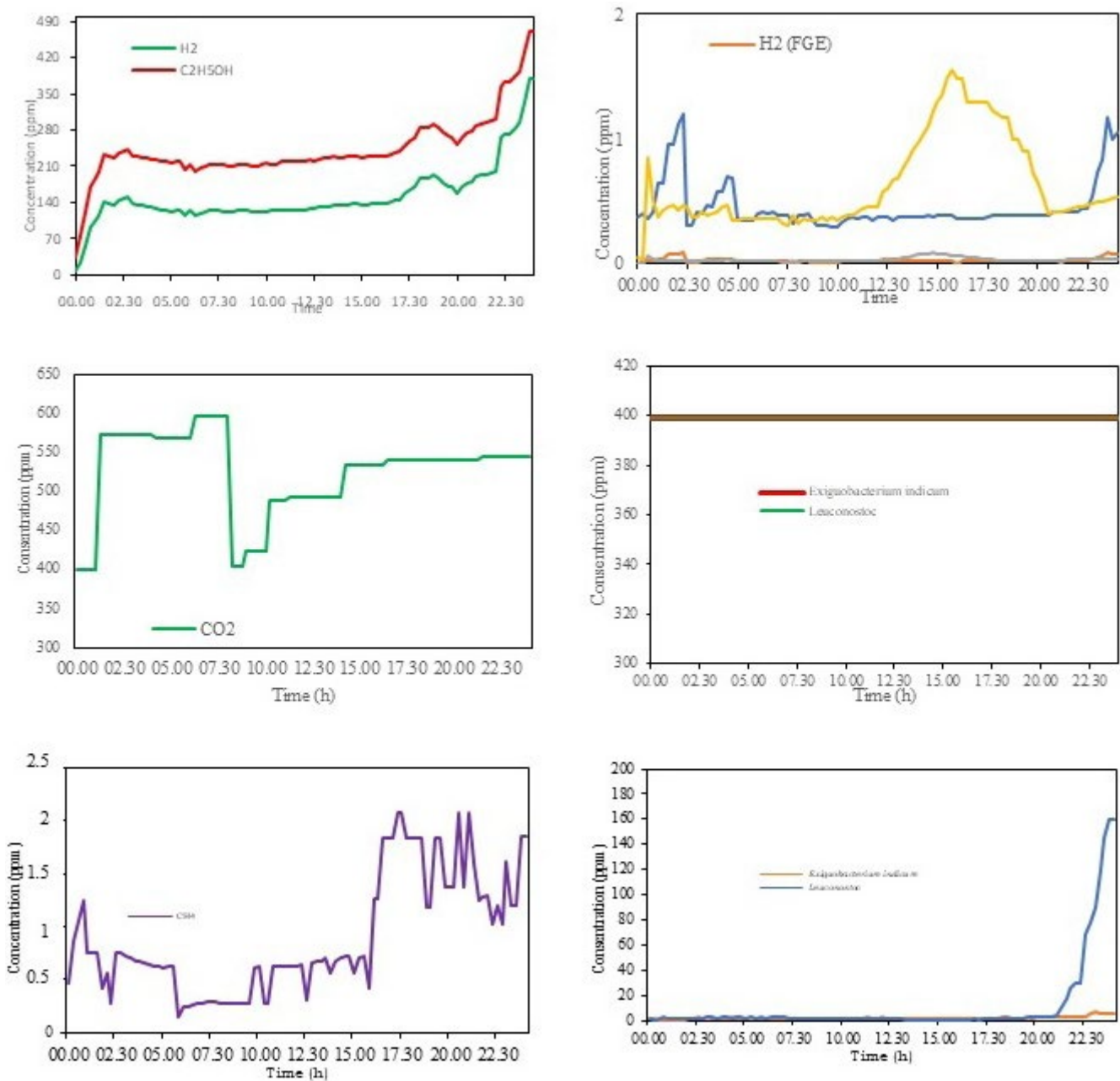


Figure 6: Gas concentrations measured during fermentation, showing ethanol and hydrogen (H_2) in FCB (A); methane (CH_4) in FGE and FGL (B); carbon dioxide (CO_2) in FCB (C); carbon dioxide (CO_2) in FGE and FGL (D); and methane (CH_4) in FCB (purple), FGE (red), and FGL (blue) (E–F).

Methane (CH₄) generation, as detected by the MQ-4 sensor, remained below the typical atmospheric background concentration of 1.8 ppm for most treatments (Figures 6E and 6F). However, a transient increase in CH₄ was observed during fermentation with *E. indicum*, reaching a maximum of approximately 7 ppm. This minor elevation may be attributed to cross-reactivity of the sensor with trace volatile organic compounds (VOCs) or limited methanogenic activity mediated by coexisting microbial populations within the substrate.

Collectively, the gas-exhaust monitoring data reveal distinct microbial metabolic behaviors under different inoculation treatments. The results indicate that *B. cereus* exhibits the highest gas metabolic activity, producing both H₂ and CO₂ as key fermentation by-products, whereas *Leuconostoc* and *Exiguobacterium* demonstrate more stable, low-gas-output fermentation profiles. These findings underscore the influence of microbial strain selection on gaseous metabolite evolution, which may further affect the chemical and sensory development of fermented *Coffea liberica* beans.

3.4 Chemical changes profiling

Liquid Chromatography–Mass Spectrometry (LC–MS) analysis demonstrated distinct differences in the chemical profiles between non-fermented Liberica coffee (OLC) and fermented Liberica coffee (FCB). Chromatographic analysis identified six major compounds in both samples (Figure 7). Retention times (RT) were generally comparable, indicating similar elution patterns. However, the relative abundances (% area) varied considerably between OLC and FCB. In OLC, the most abundant compounds were caffeine (36.16%) and hemiarin (36.16%), followed by umbelliferone (6.21%). In FCB, umbelliferone (43.29%) and caffeine (43.29%) were dominant, followed by p-aminobenzoic (6.33%). However, hemiarin decreased markedly compared to OLC, reflecting degradation or transformation during the fermentation process.

Chlorogenic acid was relatively low in both samples but increased slightly in FCB (2.78%) compared to OLC (1.72%), suggesting partial release or preservation during microbial activity. 4-oxo-retinoic acid showed a slight decrease in FCB. Chromatographic peaks further confirmed these shifts. In OLC, the profile was dominated by a major peak at RT 4.47 min (~54.8% area), with secondary peaks at RT 17.16 min (1.96%) and RT 18.20 min (1.46%). In contrast, FCB displayed a dominant peak at RT 5.15 min (~62.9% area), along with additional peaks at RT 14.27 min (1.03%), RT 15.45 min (1.61%), and RT 18.37 min (1.40%).

Overall, fermentation maintained the predominance of umbelliferone and caffeine while modulating the relative proportions of phenolic and retinoid derivatives. The presence of enriched mid-retention time metabolites in FCB indicates

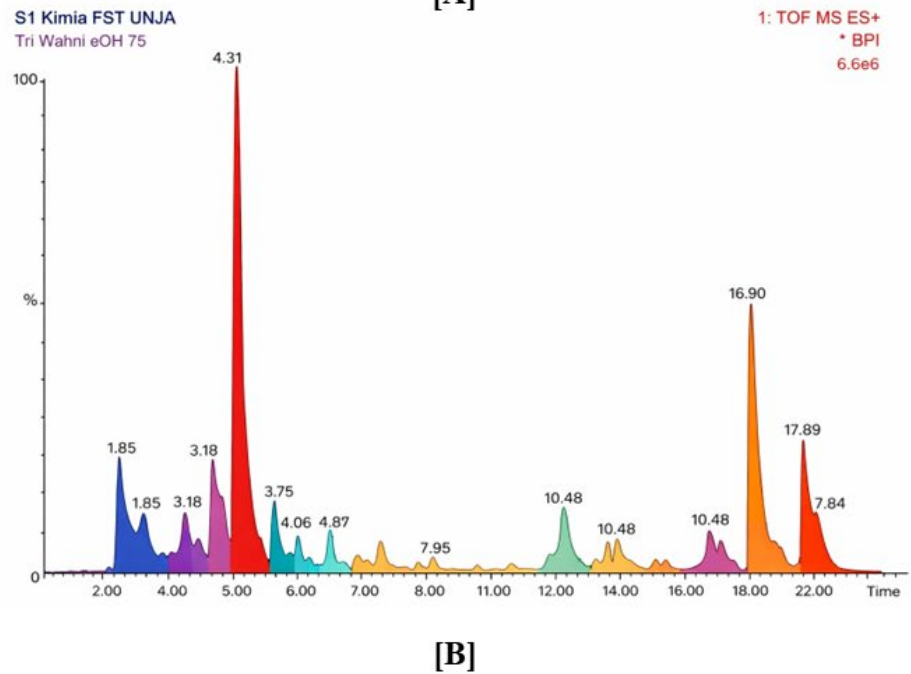
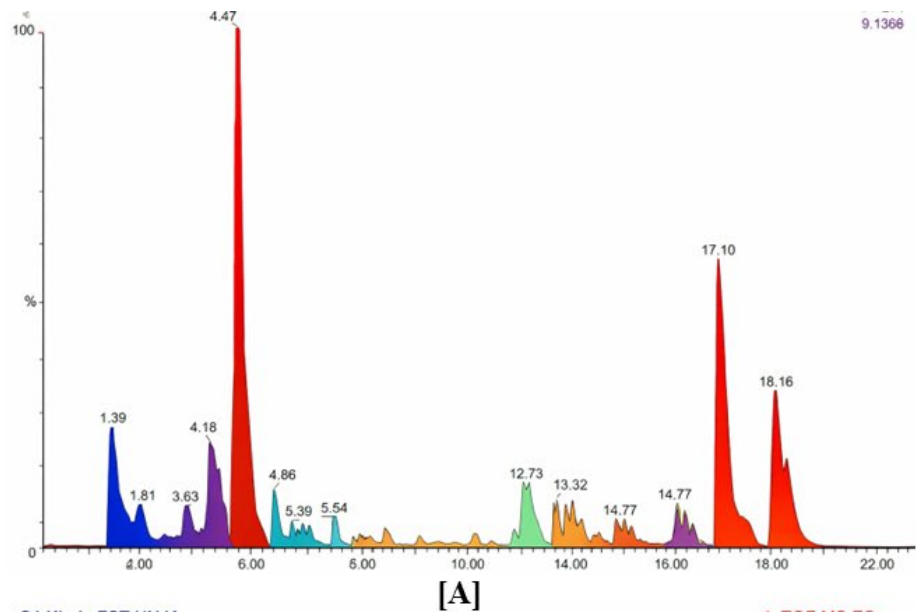
active microbial transformation involving hydrolysis, oxidation–reduction, and secondary metabolite biosynthesis. These biochemical changes suggest that fermentation not only reshapes the chemical fingerprint of Liberica coffee but may also contribute to modified sensory characteristics, enhanced bioactivity, and improved nutritional potential.

The LC–MS analysis revealed distinct alterations in the metabolite composition of *Coffea liberica* following microbial fermentation with *Bacillus cereus*. The enrichment of umbelliferone and the concurrent reduction in hemiarin content suggest active biotransformation of phenolic precursors during fermentation. These chemical changes are consistent with reports by Lee et al. (2015) and De Melo Pereira et al. (2019), who observed similar conversion patterns of coumarin derivatives and phenolic esters during controlled coffee fermentations involving lactic acid bacteria and *Bacillus* spp. Such transformations typically arise from hydrolytic cleavage of glycosidic bonds and oxidation–reduction reactions, leading to the release of low-molecular-weight aromatic compounds that enhance sensory perception and flavor complexity.

3.5 Sensory Profile

Cupping scores were compared with our previous results reported by Tarigan et al. (2024) (Table 2 and Figure 8). The sensory quality of coffee fermented in the mini bioreactor (FCB) was lower than that of other samples (Figure 8). Panelists noted high uniformity, with a perfect score (10.00), reflecting consistent roasting and a stable cup profile. The aroma was dominated by chocolate, spice, and brown sugar. Flavor attributes were strongest when the coffee was hot, showing notes of chocolate, black forest, and black tea. However, as the coffee cooled, the sweetness diminished, leaving only a faint black tea note. The aftertaste was pleasant at warm temperatures but became less favorable when cool, dominated by bitterness with only a fleeting sweetness. Acidity was very low, which was unexpected, since fermentation is generally associated with increased acidity that enhances flavor complexity. The body was balanced when hot but developed a sour character as the cup cooled.

Overall, the cupping results revealed both expected and unexpected findings. As anticipated, fermentation produced a uniform and consistent cup, and the dominance of chocolate–spice–brown sugar notes was in line with previous observations in Liberica coffee. However, the very low acidity and declining sweetness during cooling were surprising outcomes, contrasting with typical fermentation-driven profiles. These unexpected results suggest that the specific microbial dynamics in the mini bioreactor might favor metabolite pathways that preserve or enhance body and bitterness rather than acidity and sweetness.



No	RT (min)		% Area		Formula	Name
	OLC	FCB	OLC	FCB		
1	1.87	1.43	5.44	6.33	C ₇ H ₇ NO ₂	p-Aminobenzoic acid
2	4.04	3.62	1.72	2.78	C ₁₅ H ₁₈ O ₉	Chlorogenic acid
3	4.27	4.27	6.21	43.29	C ₉ H ₈ O ₃	Umbelliferone
4	5.13	4.47	36.16	43.29	C ₈ H ₁₁ N ₄ O ₂	Caffeine
5	5.34	5.14	36.16	2.22	C ₁₀ H ₈ O ₃	Hemiarin
6	8.39	7.71	3.48	1.40	C ₂₀ H ₂₆ O ₃	4-oxo-Retinoic acid

Figure 7: LC–MS spectra of fermented (A) and unfermented (B) *Coffea liberica* samples. (C) Retention times and peak areas of the five major metabolites identified in both samples.

Table 2: Sensory evaluation of Liberica Coffee fermented in a mini bioreactor.

Parameter	Samples (Score)		
	OLC	KBC*	FCB
Overall	7.67	8.25	7.67
Aroma	7.58	8.20	7.58
Flavor	7.67	7.75	7.67
Aftertaste	7.67	8.00	7.50
Acidity	7.77	8.00	7.43
Body	7.67	8.00	8.00
Balance	7.67	8.00	7.42
Uniformity	8.00	10.00	10.00
Clean cup	8.00	10.00	10.00
Sweetness	8.00	10.00	10.00
Final Score	77.7	86.0	83.02

Notes: OLC* = Original Liberica Coffee (this study)., KBC* = Liberica Coffee fermented with *Bacillus subtilis* (Tarigan et al., 2024), FCB = Liberica Coffee fermented in a mini bioreactor (this study).

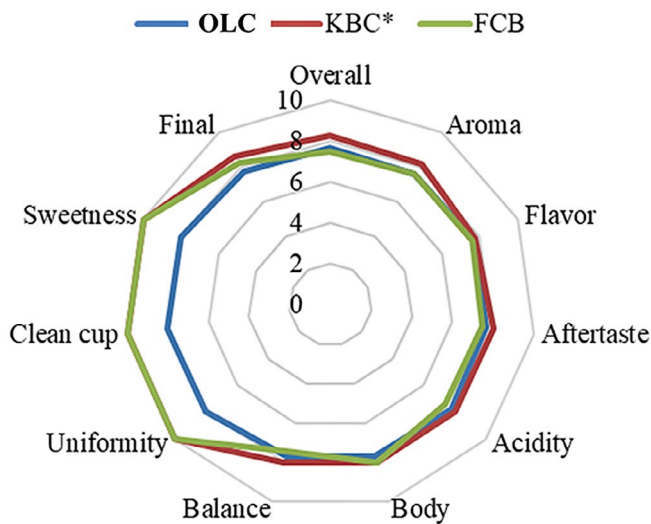


Figure 8: Sensory profile of original Liberica coffee (OLC), Liberica Coffee fermented with *Bacillus subtilis* (KBC), and Liberica Coffee fermented in a mini bioreactor (FCB).

4 DISCUSSION

4.1 Monitoring chemical reactions during Liberica Coffee fermentation

Monitoring pH and temperature provided dynamic information on microbial activity and biochemical alterations during coffee fermentation. The pH acidification observed in FCB (Figure 4A) aligns with previous reports that organic acid production is typical in coffee fermentation, contributing to spoilage control and flavor development (De Bruyn et al., 2017; Lee et al., 2015). In contrast, the stable pH in FGE and FGL (Figure 4B) may indicate either limited microbial

activity or stronger buffering capacity, reflecting matrix-specific differences. Temperature dynamics further supported these interpretations. Fluctuations in FCB temperature (Figure 5A) are consistent with metabolic heat, a typical indicator of active fermentation (Silva et al., 2024). These results highlight that both pH and temperature are not only indicators of fermentation progress but also drivers of microbial succession and metabolite formation. This has direct implications for controlling fermentation outcomes and the sensory quality of Liberica coffee.

The gas profiles observed during fermentation were consistent with patterns of microbial metabolic activity (Figure 6). The lag phase of fermentation had lower gas levels. Miñón-fuentes and Aguilar-juárez (2019) described this phase as the period in which microorganisms adjust to environmental conditions such as pH, temperature, oxygen availability, and nutrient composition before initiating active metabolism. Microbial fermentation typically increases CO₂ and H₂ production under optimal acidic conditions, especially during the exponential growth phase (Moreno Cárdenas et al., 2020; Neto et al., 2020; Renaudie et al., 2024). However, no significant H₂ production occurred in FGL and FGE (Figure 6B). This absence may result from two factors: (1) technical issues, including sensor degradation (e.g., damage to the MG811 module) and current leakage, and (2) biological limitations, such as the lack of fermentable substrates in green beans and the absence of mucilage, both of which are necessary to sustain hydrogenogenic pathways. Renaudie et al. (2024) emphasized that H₂ generation depends strongly on substrate richness, especially the availability of complex carbohydrates, hemicellulose, and lignocellulosic material, which act as hydrogen donors during fermentation. Furthermore, the shift toward neutral pH at the end of

fermentation in certain treatments (particularly those without mucilage) suppressed hydrogen formation. This agrees with Moreno Cárdenas et al. (2020), who reported that hydrogenase enzymes are pH-sensitive and function optimally under mildly acidic environments.

The low methane production indicates minimal methanogenic activity, which is expected since *B. cereus* is a facultative anaerobe that does not participate in methanogenesis (Figure 6 E and F). The small CH₄ peak may be due to contamination with methanogenic archaea or cross-feeding among microbial groups. Zhang et al. (2021) explained that CH₄ formation during anaerobic digestion occurs through four sequential steps: hydrolysis, acidogenesis, acetogenesis, and methanogenesis. In systems where volatile fatty acids and H₂ accumulate, methanogens can convert these intermediates into CH₄ and CO₂ under strict anaerobic conditions and in the presence of suitable microbial consortia.

Collectively, these findings highlight the importance of substrate composition, microbial consortium selection, and precise sensor calibration in bioreactor-based coffee fermentation systems.

4.2 LC-MS Profile

The LC-MS analysis of *Liberica* coffee fermented with *B. cereus* (FCB) revealed a diverse metabolite profile. Caffeine were the predominant compounds in both unfermented *Liberica* coffee (OLC) and FCB, each representing ~43% of the total chromatographic area. This supports previous findings reporting that caffeine remains stable during fermentation, with its abundance largely determined by microbial strains and fermentation conditions (De Bruyn et al., 2017) (De Bruyn et al., 2017; Lee et al., 2021). Interestingly, the chlorogenic acid content increased slightly in FCB (2.78%) compared with OLC (1.72%), indicating that fermentation may help preserve or even enhance specific phenolic compounds. Conversely, herniarin levels decreased significantly after fermentation, suggesting that certain non-essential metabolites may be degraded during the process. These findings are consistent with previous reports on controlled fermentations, where selective modulation of phenolic metabolites not only maintains but also enhances the antioxidant potential of the final product (Pereira et al., 2017).

Minor compounds such as p-aminobenzoic acid, and 4-oxo-retinoic acid contributed less than 7% of the total chromatographic area in both coffee types. Among these, p-aminobenzoic acid increased slightly after fermentation, whereas herniarin, and 4-oxo-retinoic acid decreased substantially, potentially due to degradation or structural transformation during microbial activity, as reported in post-harvest bioactive compound analyses (Ollennu-Chuasam et al., 2024). These shifts suggest that while fermentation in *Liberica* coffee preserves dominant metabolites such as umbelliferone

and caffeine, it selectively modulates minor constituents. This agrees with previous findings that fermentation alters the coffee chemical profile without compromising key bioactive components (Galarza & Figueroa, 2022). Interestingly, no significant caffeine degradation was observed, indicating that *B. cereus* does not metabolize methylxanthines under the tested conditions, consistent with Pisponen and Andreson (2024). Overall, the LC-MS fingerprint of fermented *Liberica* coffee shows a complex and enriched chemical profile, with specific biomarkers pointing to active microbial metabolism and potential sensory improvement. Further studies are needed to directly link these chemical shifts to flavor and aroma.

The observed increase in umbelliferone concentration in FCB implies that *B. cereus* may catalyze hydrolytic degradation of hemiarin or related precursors, yielding coumarin-type metabolites with known antioxidant and antimicrobial properties (Daglia, 2012). These metabolites have been associated with improved cup aroma, mild sweetness, and enhanced smoothness, aligning with the sensory outcomes reported in the cupping analysis. Meanwhile, the moderate rise in chlorogenic acid levels indicates partial preservation or enzymatic liberation of bound phenolic acids during fermentation, corroborating findings by Dawidowicz and Typek, (2017) and Elhalis et al. (2020), who demonstrated that microbial esterases can prevent extensive degradation of chlorogenic acid under mild fermentation conditions.

Furthermore, the alteration in chromatographic peaks—specifically the shift from RT 4.47 min in OLC to RT 5.15 min in FCB—reflects formation of new intermediate metabolites and a redistribution of existing compound pools. This phenomenon is frequently observed in microbial transformations of coffee and cacao substrates, where enzymatic oxidation of phenolic compounds leads to the generation of secondary metabolites with enhanced sensory and functional properties (Abd-Alrahman et al., 2023). Such shifts not only reshape the chemical fingerprint of the coffee matrix but also contribute to its increased bioactive potential.

The overall pattern of metabolite modulation in FCB, characterized by higher levels of umbelliferone and caffeine and reduced hemiarin, suggests a selective metabolic adaptation by *Bacillus cereus*. This bacterium is known to produce extracellular oxidoreductases and hydrolases capable of catalyzing aromatic ring modifications and phenolic decarboxylation reactions, which influence both flavor and antioxidant capacity (Contreras-Jáquez et al., 2021; Li et al., 2021). The metabolic consistency observed between the LC-MS data and the sensory evaluation supports a strong link between microbial biochemical pathways and the development of distinctive cup quality attributes.

Collectively, these results align with previous findings that controlled microbial fermentation enhances the chemical and sensory profiles of coffee through regulated

biotransformation of bioactive metabolites (Araujo et al., 2021; De Melo Pereira et al., 2019). The synergistic interaction between microbial metabolism and coffee substrate chemistry thus appears to underlie the improved aroma, acidity balance, and overall cup quality observed in the fermented *Coffea liberica* samples.

4.3 Fermentation changes sensory profile

The sensory evaluation results (Table 2, Figure 8) demonstrated distinct differences among the three *Coffea liberica* samples. The control sample (OLC) exhibited a balanced and clean cup profile with moderate acidity and sweetness, while the coffee fermented with *Bacillus subtilis* (KBC) achieved the highest overall score (86.0), corresponding to an “excellent” cup according to SCAA standards. In contrast, the sample fermented with *Bacillus cereus* (FCB) displayed a lower final score (83.02), although it still fell within the “very good” category.

The slightly lower sensory quality of the FCB may be partially attributed to the roasting profile, which was moderately dark. Darker roasting is known to reduce perceived acidity and sweetness due to thermal degradation of organic acids and Maillard-derived sugar compounds, while enhancing bitterness and body (Cruz-O’byrne et al., 2021; Sharma et al., 2020). The lower acidity (7.43) and sweetness (8.00) observed for FCB compared to KBC are consistent with this thermal modulation effect. In addition, the microbial fermentation step itself may have altered the precursor availability for volatile compound formation, leading to a different balance of aromatic compounds during roasting.

From a biochemical perspective, the LC–MS data support these sensory findings. The FCB sample exhibited a higher relative abundance of umbelliferone and caffeine, but a reduction in hemiarin and retinoid derivatives, indicating selective microbial biotransformation during fermentation. Umbelliferone, a coumarin derivative, contributes floral and honey-like notes but can suppress fruity acidity when present in excess. Conversely, *Bacillus cereus* fermentation may not generate the same range of ester or aldehyde volatiles as observed in *Bacillus subtilis*-fermented coffee (Contreras-Jáquez et al., 2021; Li et al., 2021), which could explain the lower aroma and flavor intensity scores of FCB. Moreover, the gas monitoring data revealed higher CO₂ and H₂ generation in FCB, reflecting more active carbohydrate degradation and potential formation of organic acids. While moderate acidification enhances complexity, excessive acid production can yield off-flavors or astringency. The lower pH recorded for FCB during mid-fermentation supports this explanation, suggesting a microbial metabolism skewed toward acidogenesis rather than balanced flavor precursor synthesis.

Despite its slightly reduced acidity and brightness, the FCB maintained high scores for clean cup, uniformity, and

balance (10.00), indicating that fermentation did not introduce microbial off-flavors or undesirable defects. This demonstrates that *B. cereus* can be safely utilized under controlled conditions and may impart unique aromatic subtleties while maintaining cup cleanliness. However, achieving optimal sensory quality likely requires fine-tuning of fermentation time, microbial load, and roasting intensity to preserve desirable acidity and sweetness.

Overall, these results suggest that while microbial fermentation significantly modifies the chemical and sensory matrix of *Coffea liberica*, the choice of bacterial strain and subsequent roasting profile play decisive roles in determining final cup characteristics. Controlled bioprocess design — integrating microbial selection, process monitoring, and post-fermentation roasting — thus represents a viable strategy for tailoring the flavor profile and quality consistency of specialty *Liberica* coffee.

5 CONCLUSIONS

This study demonstrates that a custom-built stainless-steel mini bioreactor enables stable, real-time monitoring of *Coffea liberica* fermentation and effective microbial control. *Bacillus cereus* exhibited the highest metabolic activity, driving significant pH reduction, temperature increase, and CO₂ production, alongside notable biochemical transformations confirmed by LC–MS. These changes influenced the sensory profile, yielding clean cup characteristics. Overall, controlled fermentation using *B. cereus* offers a promising strategy for precision coffee processing and flavor innovation.

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7 AUTHOR CONTRIBUTION

Conceptual Idea: Sutrisno, S; Tarigan, I.L; M.F. Afriyanto; Methodology design: Sutrisno, S; Tarigan, I.L; M.F. Afriyanto; Data collection: Ilham, A.K; Tarigan, I.L.; Ramadhan, I.I; Data analysis and interpretation: Ilham, A.K; Tarigan, I.L; Latief, M; Afriyanto, M.F; and Writing and editing: Ilham, A.K; Tarigan, I.L; Sutrisno, S.

8 DATA AVAILABILITY STATEMENT

Data available upon request to authors.

9 REFERENCES

- Abd-Alrahman, A. M et al. (2023). Production of natural flavor compounds using *Bacillus subtilis*-fermented soybean meal extract and their biological potential: a comprehensive in vitro study. *Frontiers in Nutrition*, 10:1280209.
- Araujo, J. B. S. et al. (2021). Decomposition and nutrients released from forest and perennial crops associated with organic coffee. *Coffee Science*, 16:e161845.
- Banti, M., & Atlaw, T. (2024). Effect of processing methods on physicochemical and cup quality of coffee at Jimma, Ethiopia. *Heliyon*, 10(9):e30480.
- Bressani, A. P. P. et al. (2018). Characteristics of fermented coffee inoculated with yeast starter cultures using different inoculation methods. *LWT*, 92:212-219.
- Cheserek, J. J. et al. (2021). Green bean biochemical attributes of Arabusta coffee hybrids from Kenya using HPLC and soxhlet extraction methods. *Australian Journal of Crop Science*, 15(2):201-208.
- Contreras-Jáquez, V. et al. (2021). In-cell crosslinked enzymes: improving *Bacillus megaterium* whole-cell biocatalyst stability for the decarboxylation of ferulic acid. *Process Biochemistry*, 110:71-84.
- Cruz-O'byrne, R. et al. (2021). Microbial diversity associated with spontaneous coffee bean fermentation process and specialty coffee production in northern Colombia. *International Journal of Food Microbiology*, 354:109282.
- Daglia, M. (2012). Polyphenols as antimicrobial agents. *Current Opinion in Biotechnology*, 23(2):174-181.
- Dawidowicz, A. L., & Typek, R. (2017). Transformation of chlorogenic acids during the coffee beans roasting process. *European Food Research and Technology*, 243(3):379-390.
- De Bruyn, F. et al. (2017). Exploring the impacts of postharvest processing on the microbiota and metabolite profiles during green coffee bean production. *Applied and environmental microbiology*, 83(1):1-16.
- De Carvalho Neto, D. P. et al. (2018). Efficient coffee beans mucilage layer removal using lactic acid fermentation in a stirred-tank bioreactor: Kinetic, metabolic and sensorial studies. *Food Bioscience*, 26:80-87.
- De Melo Pereira, G. V. et al. (2019). Exploring the impacts of postharvest processing on the aroma formation of coffee beans - A review. *Food Chemistry*, 272:441-452.
- Elhalis, H. et al. (2020). The crucial role of yeasts in the wet fermentation of coffee beans and quality. *International Journal of Food Microbiology*, 333:108796.
- Elhalis, H., Cox, J., & Zhao, J. (2023). Coffee fermentation: Expedition from traditional to controlled process and perspectives for industrialization. *Applied Food Research*, 3:100253.
- Galarza, G., & Figueroa, J. G. (2022). Volatile compound characterization of coffee (*Coffea arabica*) processed at different fermentation times using SPME-GC-MS. *Molecules*, 27(6):2004.
- Hadipernata, M.; & Nugraha, S. (2018). Process technology of luwak coffee through bioreactor utilization. *IOP Conference Series: Earth and Environmental Science*, 102:012092.
- International Coffee Organization - ICO. (2022). *Annual Review Coffee Year 2021/2022*. . <https://ico.org/annual-review/>
- Jumhawan, U. et al. (2015). Application of gas chromatography/flame ionization detector-based metabolite fingerprinting for authentication of Asian palm civet coffee (Kopi Luwak). *Journal of Bioscience and Bioengineering*, 120(5):555-561.
- Jumhawan, U. et al. (2013). Selection of discriminant markers for authentication of asian palm civet coffee (Kopi Luwak): A metabolomics approach. *Journal of Agricultural and Food Chemistry*, 61(33):7994-8001.
- Latief, M. et al. (2023). Coffee improvement by wet fermentation using *Lactobacillus plantarum*: sensory studies, proximate analysis, antioxidants, and chemical compounds. *Jurnal Rekayasa Kimia & Lingkungan*, 18(2):134-148.
- Lee, L. W. et al. (2015). Coffee fermentation and flavor - An intricate and delicate relationship. *Food Chemistry*, 185:182-191.
- Li, Qin et al. (2021). Improving the catalytic characteristics of phenolic acid decarboxylase from *Bacillus amyloliquefaciens* by the engineering of N-terminus and C-terminus. *BMC Biotechnology*, 21(1):44.
- Magalhães Júnior, A. I. et al. (2021). A critical techno-economic analysis of coffee processing utilizing a modern fermentation system: Implications for specialty coffee production. *Food and Bioprocess Technology*, 125:14-21.

- Martinez, S. J. et al. (2021). Novel stainless steel tanks enhances coffee fermentation quality. *Food Research International*, 139:109921.
- Miñón-Fuentes, R., & Aguilar-Juárez, O. (2019). Hydrogen production from coffee pulp by dark fermentation. *Water science and technology: A journal of the International Association on Water Pollution Research*, 80(9):1692-1701.
- Moreno Cárdenas, E. L. et al. (2020). Modeling dark fermentation of coffee mucilage wastes for hydrogen production: artificial neural network model vs. fuzzy logic model. *Energies*, 13(7):1663.
- Neto, D. P. de C. et al. (2020). Microbiological , physicochemical and sensory studies of coffee beans fermentation conducted in a yeast bioreactor model. *Food Biotechnology*, 34(2):172-192.
- Ollennu-Chuasam, P. et al. (2024). Lipophilic and hydrophilic metabolites as descriptors of different coffee beverages. *Journal of agricultural and food chemistry*, 72(29):16461-16474.
- Pereira, G. V. de M. et al. (2017). Microbial ecology and starter culture technology in coffee processing. *Critical Reviews in Food Science and Nutrition*, 57(13):2775-2788.
- Pisponen, A., & Andreson, H. (2024). The impact of lactic acid bacteria and yeasts ratio on fermentation and taste of kvass. *Agronomy Research*, 22:513-522.
- Putri, S. P. et al. (2015). Application of GC / MS and GC / FID-based metabolomics for authentication of Asian palm civet coffee (Kopi Luwak). *Metabolomics*, 3(2):34-41
- Renaudie, M. et al. (2024). Spontaneous dark fermentation in a pre-seeded liquid-gas membrane bioreactor: Impact of wine and coffee biowaste microflora on continuous biohydrogen production. *Journal of Cleaner Production*, 437:140759.
- Reyes, E. I. M. et al. (2024). Controlled fermentation in artisanal bioreactors to produce specialty coffees. *Ciência e Agrotecnologia*, 48:e007524.
- Salengke, S. et al. (2019). Technology innovation for production of specialty coffee. *IOP Conference Series: Earth and Environmental Science*, 355:012105.
- Shah, P. et al. (2024). Chapter 9 - Anaerobic fermentation for biogas production. In M. Jeguirim., & A. A. Zorpas, (Eds.) *Advances in biofuels production, optimization and applications*. [S.l.]: Elsevier, (pp.165-183).
- Sharma, R. et al. (2020). Microbial Fermentation and Its Role in Quality Improvement of Fermented Foods. *Fermentation*, 6(4):106.
- Silva, L. C. F. et al. (2024). Enhancing sensory quality of coffee: the impact of fermentation techniques on *Coffea arabica* cv. Catiguá MG2. *Foods*, 13(5):653.
- Somporn, C. et al. (2011). Effects of roasting degree on radical scavenging activity, phenolics and volatile compounds of Arabica coffee beans (*Coffea arabica* L. cv. Catimor). *International Journal of Food Science and Technology*, 46(11):2287-2296.
- Sridevi, V. et al. (2024). Challenges and opportunities in the production of sustainable hydrogen from lignocellulosic biomass using microwave-assisted pyrolysis: A review. *International Journal of Hydrogen Energy*, 52:507-531.
- Tarigan, I. L. et al. (2024a). Changes in the chemical compound and sensory profiles of liberica fermentation (*Coffea liberica*) with Cellulolytic Bacteria *Alcaligenes* sp and *Exiguobacterium indicum*. *Coffee Science*, 19:e192258.
- Tarigan, I. L. e et al. (2024b). Enhancement of liberica coffee quality by wet fermentation using *Bacillus subtilis*. *ALCHEMY Jurnal Penelitian Kimia*, 20(2):162-177.
- Toci, A. T.; & Farah, A. (2008). Volatile compounds as potential defective coffee beans' markers. *Food Chemistry*, 108(3):1133-1141.
- Tolentino Vaz, C. J. et al. (2022). Effect of fermentation of arabica coffee on physicochemical characteristics and sensory analysis. *Research Square*, 2022:1-14.
- Towaha, J., & Rubiyo, R. (2016). Physical quality and flavor of arabica coffee beans fermented by probiotic microbes from civet digestive system. *Jurnal Tanaman Industri dan Penyegar*, 3(2):61.
- Zhang, T. et al. (2021). Improvement of coffee grounds high solid thermophilic methane fermentation by co-digestion with in-situ produced waste activated sludge: Performance and stability. *Science of The Total Environment*, 765:142551.