

Comparison of Chemical Changes in Controlled Fermentation of Liberica Coffee (*Coffea liberica*) Using *Lactobacillus plantarum*, *Aspergillus niger*, and *Saccharomyces cerevisiae*

Indra Lasmana Tarigan^{1,3}, Amanda Fitri Rahmawati², Salmiyah Oktariyani², Madyawati Latief^{1,3}, Noor Ariefandie Febrianto⁴ and Sutrisno Sutrisno^{1,3,*}

¹Department of Chemistry, Faculty of Science and Technology, Universitas Jambi, Jambi, Indonesia

²Undergraduate Student, Department of Chemistry, Faculty of Science and Technology, Universitas Jambi, Jambi, Indonesia

³The University Centre of Excellences E2-KOLIM, Universitas Jambi, Jambi, Indonesia

⁴Indonesian Coffee and Cocoa Research Institute (ICCRI), PT. Riset Perkebunan Nusantara, Jember, Indonesia

(*Corresponding author's e-mail: herasutrisno@unja.ac.id)

Received: 17 February 2026, Revised: 17 April 2026, Accepted: 30 April 2026, Published: 5 June 2026

Abstract

Liberica coffee (*Coffea liberica*), which accounts for approximately 2% of global coffee production, has received limited attention due to its relatively lower sensory quality compared to Arabica. This study investigated the chemical and sensory changes of Liberica coffee subjected to controlled fermentation in a bioreactor system. Green beans were fermented separately (single-strain fermentation) for 24 h using *Lactobacillus plantarum*, *Aspergillus niger*, and *Saccharomyces cerevisiae* under controlled conditions, with non-fermented samples as controls and experiments conducted in triplicate. Fermentation progress was monitored through pH and temperature profiles, while gas production (primarily CO₂ and alcohol vapor) was used as an indicator of microbial metabolic activity. The fermented beans were subsequently medium-roasted and evaluated using cupping tests, LC-MS analysis, and DPPH antioxidant assays. Statistical differences were assessed using ANOVA ($p < 0.05$). Fermented samples showed significantly improved sensory scores (up to 79.25, “very good” category) compared to non-fermented coffee (72.5). Antioxidant activity was also enhanced, with lower IC₅₀ values (as low as 33.16 ppm), and a greater diversity of bioactive compounds was observed (up to 18 compounds vs. 8 in non-fermented samples). Among the tested microorganisms, *Aspergillus niger* produced the best sensory performance, *Saccharomyces cerevisiae* yielded the highest diversity of bioactive compounds, and both *S. cerevisiae* and *L. plantarum* showed strong antioxidant activity. These findings demonstrate that controlled microbial fermentation is a promising and reproducible approach to enhance the sensory quality, chemical diversity, and functional properties of Liberica coffee, providing a basis for developing targeted fermentation protocols in specialty Liberica coffee processing.

Keywords: Liberica coffee, Controlled fermentation, Bioreactor, Bioactive compounds, Antioxidant activity, Microbial fermentation

Abbreviations

DPPH	: 2,2-diphenyl-1-picrylhydrazyl	PDA	: Potato Dextrose Agar
LC-MS	: Liquid Chromatography–Mass Spectrometry	PDB	: Potato Dextrose Broth
UPLC	: Ultra-Performance Liquid Chromatography	MRS	: De Man, Rogosa, and Sharpe
ESI	: Electrospray Ionization	NA	: Nutrient Agar

SCAA : Specialty Coffee Association of America

SNI : Standar Nasional Indonesia

CFU : Colony Forming Unit

UV–Vis : Ultraviolet-Visible

HMDB : Human Metabolome Database

Introduction

Coffee is one of the most economically valuable agricultural commodities worldwide and serves as a primary source of income for millions of farmers. Among commercially cultivated species, *Coffea arabica* and *Coffea canephora* (Robusta) dominate global production, whereas Liberica coffee accounts for only approximately 2% of total world output. Despite its limited contribution, Liberica coffee possesses substantial development potential, particularly in tropical regions such as Indonesia. One of the major production centers is Tanjung Jabung Barat Regency, Jambi Province, where the Liberica Tungkal Komposit variety is extensively cultivated [1,2]. Liberica coffee is characterized by its distinctive jackfruit-like flavor and its adaptability to peatland soils, as well as greater tolerance to certain plant diseases. However, its sensory quality is often perceived as less consistent compared to Arabica, which limits its broader commercial utilization.

Coffee quality is strongly influenced by post-harvest processing, particularly fermentation, as well as roasting and brewing conditions [3,4]. Fermented coffee is generally associated with more complex sensory profiles, including fruity, caramel, and nutty notes, whereas non-fermented coffee tends to exhibit flatter characteristics and higher bitterness [5,6]. However, conventional spontaneous fermentation relies on naturally occurring microorganisms and lacks process control, often resulting in inconsistent sensory attributes and undesirable flavors such as excessive acidity or over-fermented notes [7]. This variability reduces reproducibility and poses challenges for quality standardization and large-scale application.

To overcome these limitations, controlled fermentation using bioreactor systems has emerged as a promising approach. Bioreactors enable real-time monitoring and precise regulation of key parameters, including temperature, pH, dissolved oxygen, and agitation, thereby improving process consistency and reproducibility [8]. Such controlled conditions have been reported to enhance sensory quality, with Specialty Coffee Association (SCAA) scores reaching up to 84

and demonstrating improved product consistency [9]. In addition to process control, the selection of appropriate microbial strains plays a crucial role in determining fermentation outcomes. Microorganisms such as *Lactobacillus plantarum*, yeast, and filamentous fungi can influence mucilage degradation, inhibit undesirable microbes, and promote the formation of volatile and bioactive compounds that contribute to aroma and flavor development [10,11].

During fermentation, microbial metabolism converts carbohydrates and proteins into simpler compounds, including sugars and amino acids, which act as precursors for Maillard reactions during roasting and contribute to the formation of flavor-active compounds [12,13]. Roasting further modulates the composition of bioactive and antioxidant compounds through complex thermal reactions, including caramelization and the transformation of phenolic compounds such as chlorogenic acids and melanoidins [14]. Despite recent advances, several important gaps remain in the study of Liberica coffee fermentation. First, comparative studies evaluating controlled fermentation using different microbial groups—particularly lactic acid bacteria, yeast, and filamentous fungi—within a bioreactor system are still lacking. Second, the specific effects of these microorganisms on the sensory quality and proximate composition of Liberica coffee are not yet well understood. Third, integrated studies combining LC-MS-based metabolite profiling with antioxidant activity assessment in fermented Liberica coffee remain limited. Addressing these gaps is essential to better understand and optimize fermentation strategies for improving Liberica coffee quality.

Therefore, this study aimed to comparatively evaluate the effects of controlled fermentation using *Lactobacillus plantarum*, *Aspergillus niger*, and *Saccharomyces cerevisiae* in a bioreactor system on sensory characteristics, bioactive compound profiles, and antioxidant activity of Liberica coffee. It was hypothesized that different microbial groups would produce distinct metabolic transformations, with lactic

acid bacteria enhancing acidity and flavor balance, yeast improving aroma complexity, and filamentous fungi increasing compound diversity and functional properties.

Materials and methods

Materials and instrumentation

Green bean *Coffea liberica* (Grade C) were obtained from a local farmer (CV. Kopi Ajib, Dusun Sungai Haji, Sungai Terap Village, Betara District, Tanjung Jabung Barat Regency, Jambi, Indonesia; coordinates: -0.96818, 103.40691). The coffee cherries were harvested at optimal ripeness, processed into green beans, and transported to the laboratory for further experimentation. The culture media used in this study included De Man, Rogosa, and Sharpe (MRS) broth, Nutrient Agar (NA), Potato Dextrose Agar (PDA), and Potato Dextrose Broth (PDB). Additional reagents comprised distilled water (aquades), 70% ethanol, 96% ethanol, 0.9% NaCl solution, sulfuric acid (H₂SO₄), sodium hydroxide (NaOH), and a mixed indicator solution containing 0.1% methyl red and 0.2% bromocresol green. All chemicals were of analytical grade and used without further purification. Microbial strains (*Saccharomyces cerevisiae*, *Lactobacillus plantarum*, and *Aspergillus niger* were obtained from

the Indonesian Culture Collection (INACC), National Research and Innovation Agency (BRIN), Indonesia, and were maintained according to standard microbiological procedures prior to fermentation experiments.

Chemical profiling of bioactive compounds was performed using Liquid Chromatography-Mass Spectrometry (LC-MS) (ACQUITY UPLC system, Waters Corporation, Milford, MA, USA) equipped with an ACQUITY UPLC HSS column (1.8 μ m, 2.1×100 mm). Spectrophotometric measurements, including antioxidant activity assays, were conducted using an Orion AquaMate 8100 UV-Visible Spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA). Roasting was carried out using a Hinoka RCI 003 coffee roaster (Hinoka Co., Japan), and roasted beans were ground using a Ferratti Ferro coffee grinder (Ferratti, Indonesia). LC-MS data acquisition and processing were performed using MassLynx V4.1 software (Waters Corporation, USA). Putative compound identification was conducted by comparison with online databases, including ChemSpider, PubChem, MassBank, and the Human Metabolome Database (HMDB). **Figure 1** illustrates the overall workflow of the study, encompassing sample preparation, processing, chemical analysis, and data interpretation.

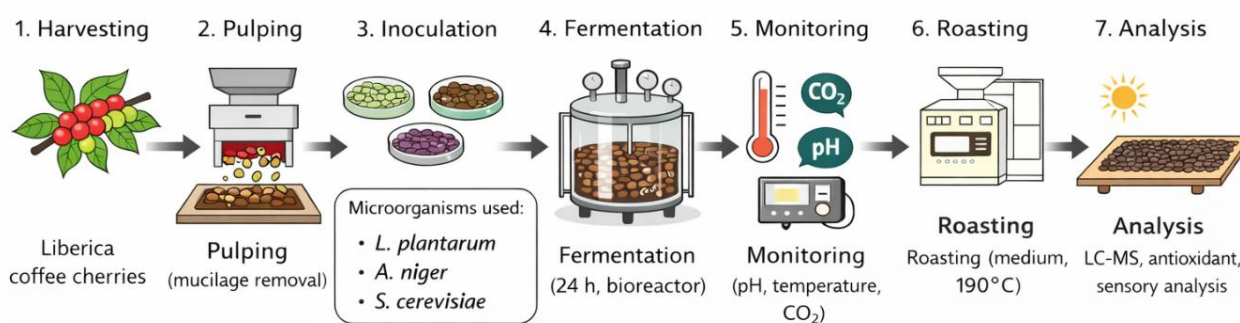


Figure 1 Process flow diagram of the production of Liberica coffee, which involves harvesting, pulping, inoculating with selected microbes (*Lactobacillus plantarum*, *Aspergillus niger*, and *Saccharomyces cerevisiae*), fermenting for 24 h in a controlled environment (pH, temperature, and CO₂), roasting at 190 °C, and analyzing using liquid chromatography-mass spectrometry (LC-MS), antioxidant analysis, and sensory evaluation.

Preparation and fermentation

All equipment and materials were cleaned, dried, and sterilized prior to experimentation. Heat-resistant apparatus was autoclaved at 121 °C for 15 min, while heat-sensitive materials were disinfected with 70%

ethanol and flame-sterilized. Microbial growth media were prepared under aseptic conditions. Nutrient Agar (5.6 g/100 mL distilled water) was heated, sterilized at 121 °C for 15 min, poured into Petri dishes, and inoculated with bacterial isolates followed by incubation

at 37 °C for 24 h to obtain stock cultures [15]. Potato Dextrose Agar (3.9 g/100 mL distilled water) was similarly sterilized, dispensed into slanted tubes, inoculated with fungal and yeast strains, and incubated at 37 °C for 24 h to establish pure cultures [16].

For starter preparation, 26.1 g MRS broth was dissolved in 500 mL distilled water, sterilized, and inoculated with *Lactobacillus plantarum*. The culture was incubated at 37 °C for 24 h, and 0.8 mL of the active suspension was transferred into 500 mL fresh MRS broth followed by further incubation for 24 h to obtain the fermentation inoculum. For *Saccharomyces cerevisiae* and *Aspergillus niger*, 24 g Potato Dextrose Broth (PDB) was dissolved in 500 mL distilled water, sterilized at 121 °C for 15 min, inoculated, and incubated under identical conditions to produce active starter cultures [17].

A total of 1 kg of Liberica green coffee beans sourced from Betara, Tanjung Jabung Barat, Jambi Province, Indonesia, were sorted and fermented separately with *Lactobacillus plantarum*, *Aspergillus niger*, and *Saccharomyces cerevisiae* for 24 h in a mini-bioreactor system (Table 1). Each 1 kg fermentation

batch was considered as one experimental unit, and all treatments were conducted in triplicate (n = 3 independent fermentation batches per microorganism). Each treatment received 500 mL of the respective starter culture to ensure complete immersion of the beans. Prior to fermentation, the inoculum concentration was standardized to ensure comparable initial microbial loads, approximately 10⁷ CFU/mL for *L. plantarum* and *S. cerevisiae*, and 10⁶ spores/mL for *A. niger*. Prior to fermentation, the closed aluminum bioreactor vessel was sterilized using UV-C irradiation for 15 - 30 min. During fermentation, pH and solution sensors were immersed directly into the substrate, and the system was connected to a 24 V power supply and interfaced with a laptop via USB. Process parameters were recorded automatically every 30 min with a 1-min acquisition interval and transferred in real time to Microsoft Excel using the Data Streamer feature. After 24 h, fermentation was terminated, and the beans were removed, washed thoroughly, and sun-dried to approximately 12% moisture content prior to further analysis [18].

Table 1 Microbial cultures utilized for fermented coffee and their associated codes for fermentation. The table indicates the microorganisms utilized in each group of fermentation treatment.

Culture	Fermentation Code
<i>Lactobacillus plantarum</i>	GA1
<i>Aspergillus niger</i>	GA2
<i>Saccharomyces cerevisiae</i>	GA3

Roasting, grinding, extraction, and LC-MS analysis

Dried Liberica coffee beans were roasted using a Hinoka RCI 003 roaster under controlled conditions following ICCRI guidelines at two levels: medium (190 °C) and dark (210 °C), each for approximately 8 min, followed by immediate cooling to stabilize volatile compounds [19]. The roasting process was monitored to identify key milestones, with first crack occurring at approximately 184 - 196 °C during medium roasting. Roasting degree was further indicated by visual color changes (brown to dark brown) and estimated bean mass loss. Each roasting treatment was coded according to the fermentation microorganism and roast level, as shown in Table 2. Roasted beans were ground using a Ferratti

Ferro coffee grinder into fine and coarse particle sizes for chemical and sensory analyses, respectively. The ground samples were stored in airtight zipper-sealed plastic bags until further analysis. For bioactive compound extraction, 100 g of finely ground coffee powder was macerated with 200 mL of 96% ethanol (solid-to-solvent ratio 1:2, w/v) for 5 days at room temperature, followed by re-maceration for an additional 2 days to maximize compound recovery. The extraction was carried out in closed containers with minimal exposure to light and air to reduce oxidation. The combined filtrates were concentrated using a rotary evaporator at 40 °C to obtain a crude ethanolic extract. The extraction yield was calculated and expressed as percentage (w/w) relative to the initial sample weight.

The extract was stored in sealed containers prior to LC-MS and antioxidant analyses [20].

LC-MS analysis was conducted at the Bogor Forensic Laboratory using an ACQUITY UPLC system equipped with an ACQUITY UPLC HSS column (1.8 μ m, 2.1 $\times$ 100 mm) maintained at 50 °C. The mobile phase consisted of water with 5 mM ammonium formate (A) and acetonitrile with 0.05% formic acid (B),

delivered at a flow rate of 0.2 mL/min for 23 min. A 5 μ L sample was injected after filtration through a 0.2 μ m syringe filter. Detection was performed using a Xevo G2-S QToF mass spectrometer with electrospray ionization (ESI) operating in the 50 - 1200 m/z range. Data acquisition and processing were conducted using MassLynx V4.1 software, and metabolite identification was performed by comparison with online databases.

Table 2 Roast treatments used for each fermented coffee treatment and the codes used for roasts. This table gives the classifications of roast conditions for both medium and dark roasts for each microbe treatment as well as the control treatment.

Culture	Code	
	Medium roast	Dark roast
<i>Lactobacillus plantarum</i>	P1	P2
<i>Aspergillus niger</i>	A1	A2
<i>Saccharomyces cerevisiae</i>	C1	C3
Non fermentasi	NFM	NFD

Antioxidant activity (DPPH Method)

Fermented Liberica coffee extracts (10 mg) were dissolved in 10 mL of ethanol to prepare stock solutions, which were further diluted to obtain working concentrations of 10, 30, and 50 ppm. A DPPH stock solution was prepared by dissolving 10 mg DPPH in 10 mL ethanol, followed by dilution to 100 ppm and 50 ppm. Ascorbic acid (10 mg) was used as a standard antioxidant and prepared similarly to obtain concentrations of 10, 30, and 50 ppm. For analysis, 0.8 mL of sample or standard solution was mixed with 7.8 mL of 50 ppm DPPH solution, incubated in the dark for 30 min, and the absorbance was measured at 517 nm using a UV-Vis spectrophotometer [21].

Sensory evaluation (Cupping Test)

Sensory analysis was conducted using the cupping protocol established by the Specialty Coffee Association (SCA). Coffee samples were ground to a 20-mesh particle size and brewed by adding 150 mL of hot water (96 °C) to 10 g of ground coffee. Wet aroma was evaluated after 3 - 5 min, while overall sensory attributes—including aroma, flavor, aftertaste, acidity, body, balance, and overall impression—were assessed at the 8th minute. Sensory scores were classified as 6.00 - 6.75 (good), 7.00 - 7.75 (very good), 8.00 - 8.75 (excellent), and 9.00 - 9.75 (outstanding) [22]. The

evaluation was performed by three certified Q-graders from Lymposite Coffee CV, ensuring high reliability and consistency of sensory assessment. Each sample was evaluated in triplicate. All panelists participated voluntarily and provided informed consent prior to the evaluation. As the study did not involve medical procedures or sensitive personal data, formal ethical approval was not required.

Proximate analysis

Proximate composition of Liberica coffee samples, including moisture, ash, crude protein, crude fat, and carbohydrate contents, was determined using standard methods of the Association of Official Analytical Chemists (AOAC). Moisture content was determined by oven-drying approximately 2 g of sample at 105 °C until a constant weight was achieved. Ash content was measured by incinerating the dried sample in a muffle furnace at 600 °C for 4 - 5 h to obtain inorganic residue. Crude protein content was analyzed using the Kjeldahl method, which involves acid digestion of the sample with concentrated sulfuric acid in the presence of a mixed catalyst, followed by distillation and titration of the released ammonia. The nitrogen content was converted to crude protein using a conversion factor of 6.25. Crude fat content was determined using Soxhlet extraction with an appropriate organic solvent for approximately 16 h, followed by evaporation of the solvent and gravimetric

determination of extracted lipids. Carbohydrate content was calculated by difference, based on the measured values of moisture, ash, protein, and fat. All analyses were conducted in triplicate. The procedures were performed in accordance with AOAC standard methods [23].

Statistical analysis

All experimental data were expressed as mean $\pm$ standard deviation of three independent replicates. Statistical analysis was performed using SPSS software. Differences among treatment groups were evaluated using one-way or two-way analysis of variance (ANOVA), depending on the experimental design. When significant differences were observed, Tukey's post hoc test was applied for multiple comparisons. A significance level of $p < 0.05$ was adopted for all statistical analyses. In addition, correlation analysis (Spearman) was performed to evaluate the relationship between major compound classes and antioxidant activity (IC_{50}). Due to the semi-quantitative nature of the LC-MS data, compound class abundance was approximated based on the presence or absence of detected compounds within each class.

Results and discussion

To ensure fair comparison among fermentation treatments, the roasting degree was standardized at medium roast, thereby minimizing roasting variability and isolating the effects of fermentation.

Monitoring of pH and temperature during fermentation

pH and temperature are critical parameters regulating microbial activity and metabolite formation during fermentation. In this study, pH decreased from approximately 6.0 to $\sim$ 5.0 after 24 h, indicating active

microbial metabolism and organic acid production (**Figure 2**). This reduction reflects substrate conversion into organic acids, which contributes to acidity and flavor development, although excessively low pH may inhibit microbial activity and reduce metabolic efficiency [23]. This trend is consistent with previous studies reporting pH decline within 16 - 24 h of coffee fermentation [24-26]. Temperature also plays a key role in fermentation efficiency and product quality. The observed temperature ranged from 27 to 29 °C and increased gradually, reflecting metabolic heat generation under optimal conditions for microbial activity [27,28]. Among treatments, *Aspergillus niger* and *Saccharomyces cerevisiae* exhibited more stable temperature profiles than *Lactobacillus plantarum*, likely due to their broader thermal tolerance and more stable metabolic responses [29,30]. In contrast, *L. plantarum* showed greater temperature fluctuation, consistent with its sensitivity to environmental changes [31,32]. Overall, the concurrent decrease in pH and increase in temperature indicate active fermentation, where both parameters are closely linked to microbial growth, metabolic pathways, and the development of coffee flavor and quality.

Despite controlled conditions, slight variations in pH and temperature may have occurred during fermentation, potentially influencing microbial activity and metabolite production. Differences in thermal tolerance and pH sensitivity among microbial species may contribute to variations in fermentation dynamics and observed results. In addition, the lack of continuous real-time monitoring may limit the precision in capturing rapid changes during the fermentation process. Therefore, these factors should be considered when interpreting the relationship between fermentation conditions and coffee quality.

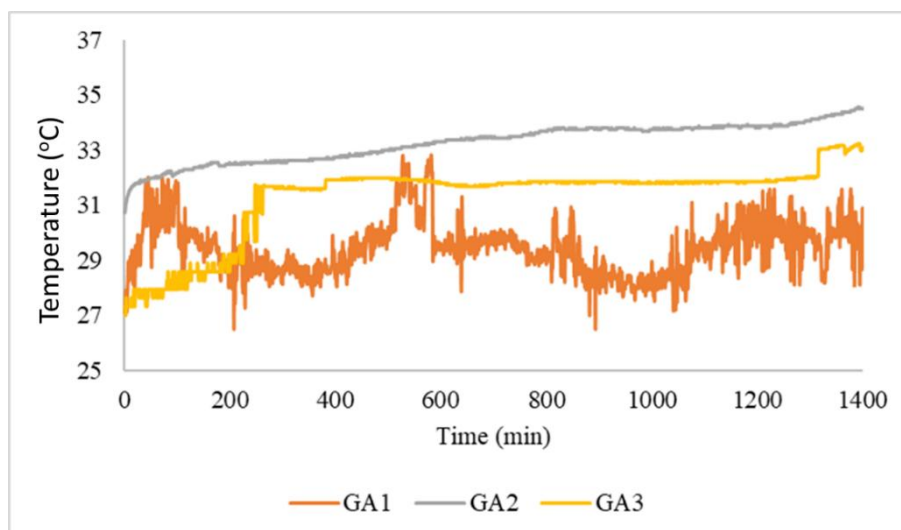


Figure 2 Changes in temperature when fermenting Liberica coffee in varying treatments (GA1, GA2, and GA3). Treatment GA2 had steady temperature changes, but GA1 had an unstable pattern of changes. In comparison, GA3 had moderate temperature change and a steady environment than GA1.

Monitoring of CO₂, Alcohol, H₂, and CH₄ production

Gas production during fermentation reflects microbial activity and metabolic pathways that influence coffee chemistry and sensory quality (**Figures 3, 4, 5 and 6**). CO₂ increased markedly in all treatments, with *Lactobacillus plantarum* rising from 657.74 ppm to 16,198.53 ppm after 24 h, followed by *Saccharomyces cerevisiae* (13,885.97 ppm) and *Aspergillus niger* (12,491.7 ppm). This pattern reflects heterofermentative and alcoholic metabolism, where carbohydrates are converted into organic acids, ethanol, and CO₂ [33-35]. CO₂ also facilitates mucilage breakdown, enhances metabolite diffusion, and may contribute to acidity through carbonic acid formation [36-39].

Alcohol production varied among microorganisms, with *S. cerevisiae* showing the highest level (11.1 ppm), followed by *L. plantarum* (4.92 ppm) and *Aspergillus sp.* (4.73 ppm). This is consistent with the strong ethanol-producing capacity of yeast via glycolysis and alcoholic fermentation [40-42]. Alcohol formation increased with fermentation time, particularly during the exponential growth phase, and contributes to aroma development through ester formation under anaerobic conditions [43].

CH₄ production was observed under anaerobic conditions, with the highest concentration in *L. plantarum* (11.21 ppm), followed by *S. cerevisiae* (10.3 ppm) and *A. niger* (6.89 ppm). This may be linked to the availability of methanogenic substrates such as H₂ and CO₂ generated during fermentation [44,45]. H₂ production further confirmed active anaerobic metabolism, with the highest level in *L. plantarum* (10.99 ppm), followed by *S. cerevisiae* (8.89 ppm) and *Aspergillus sp.* (5.96 ppm), reflecting heterofermentative pathways and hydrogenase activity [46]. H₂ production was consistent with the observed pH range (5.0 - 6.0), which is favorable for hydrogen-generating processes [47,48]. Fluctuations in H₂ levels may be associated with reduced activity of hydrogen-producing enzymes as fermentation progresses [49].

Overall, the production of CO₂, alcohol, H₂, and CH₄ highlights distinct microbial metabolic pathways that shape coffee fermentation. CO₂ influences mass transfer and acidity, alcohol contributes to aroma complexity through ester formation, while H₂ and CH₄ indicate reductive conditions that may alter the balance of organic compounds. These findings demonstrate that gas monitoring is a useful approach for understanding fermentation dynamics and optimizing coffee flavor and quality.

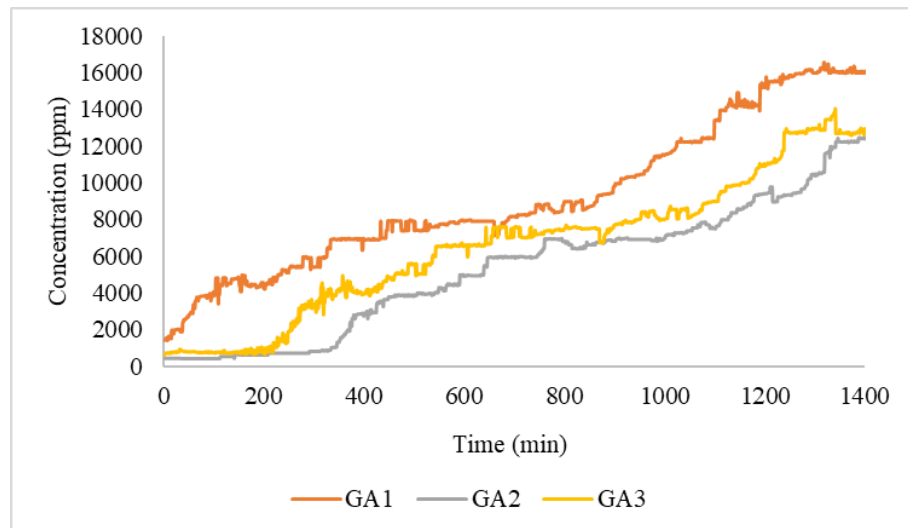


Figure 3 Production of CO₂ during fermentation of Liberica coffee using different treatments (GA1, GA2, and GA3). There was a gradual increase in CO₂ levels in all treatments, which means there was an active metabolism of microbes in all treatments. The level of CO₂ was higher in GA1 treatment than in GA3 and GA2 treatments.

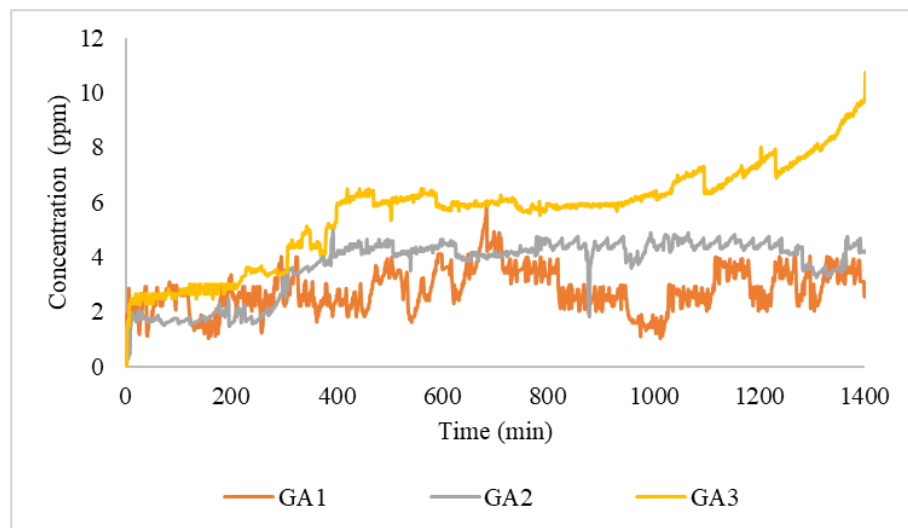


Figure 4 Production of alcohol in the fermentation of Liberica coffee under different GA1, GA2, and GA3 treatments. All treatments had increasing amounts of alcohol at various times, indicating fermentative activity. The highest alcohol content was observed in GA3 treatment, followed by GA2, whereas GA1 was relatively low.

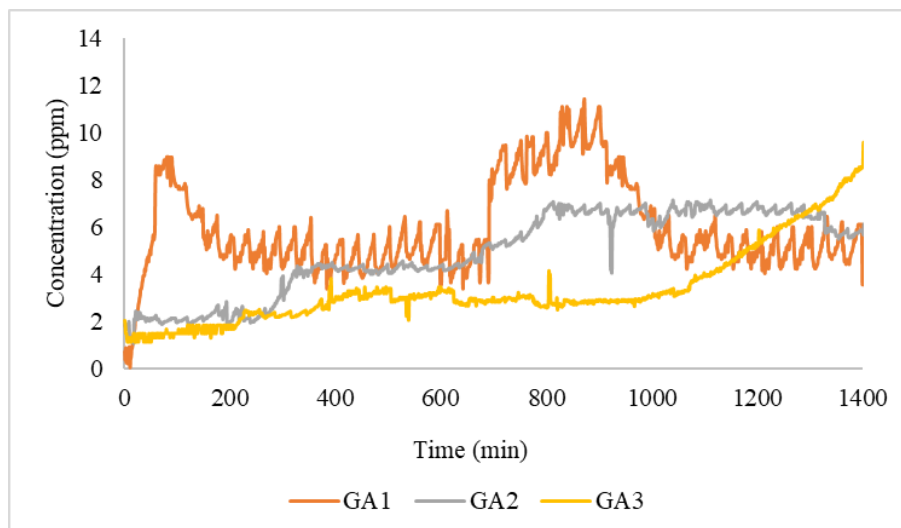


Figure 5 CH₄ generation for fermentation of coffee beans (Liberica) using various treatments (GA1, GA2, and GA3). There were significant changes in the level of methane generated in all treatment groups, but GA1 experienced rapid peaks in the earlier stage and more frequent fluctuations compared to GA2 and GA3.

Modulation of roasting profiles

Roasting is a critical processing step that significantly alters the chemical composition and sensory profile of coffee, including flavor, aroma, and bioactive potential. Thermal treatments induce a series of complex chemical reactions that generate new volatile and non-volatile compounds while simultaneously degrading some native constituents present in green coffee beans. These transformations ultimately determine the quality and character of the final beverage. During the initial stage of roasting, heat exposure promotes evaporation of moisture and low-boiling volatile compounds, causing the beans to turn yellow. As temperature increases, pyrolytic reactions intensify, accelerating moisture loss and promoting caramelization of sugars, which darkens beans color. Excessive roasting duration or temperature may lead to charring, producing blackened beans and undesirable burnt flavors [50].

Flavor development during roasting is primarily governed by Maillard reactions, Strecker degradation,

and thermal decomposition of trigonelline, amino acids, pigments, and lipids. These reactions also contribute to the formation of organic acids such as formic, acetic, glycolic, and lactic acids, which influence perceived acidity. Sucrose serves as a major precursor for many of these compounds [51]. From a physical standpoint, roasting is characterized by progressive color change from green to brown due to pyrolysis. Higher roasting degrees generally produce stronger aroma, increased bitterness, and lower acidity as a consequence of chlorogenic acid degradation and the formation of phenolic derivatives. A key milestone in roasting is the first crack, which corresponds to the rupture of the bean structure caused by internal pressure from expanding water vapor and gases. This event, followed by the second crack, is strongly influenced by initial bean moisture content and roasting conditions, since roasting is an endothermic process that requires heat transfer from the bean surface to the core [52].

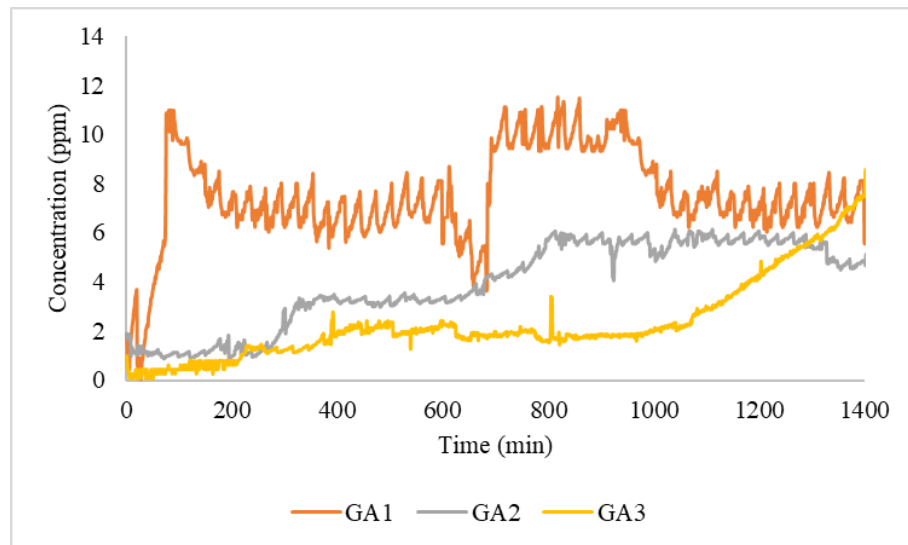


Figure 6 The production of hydrogen gas during fermentation of Liberica coffee under various conditions of GA1, GA2, and GA3. Production of hydrogen (H_2) gas under conditions GA1, GA2, and GA3 in 1400 minutes. GA1 shows fluctuations at a higher level; GA2 and GA3 show slow growth initially followed by an increase in the latter part.

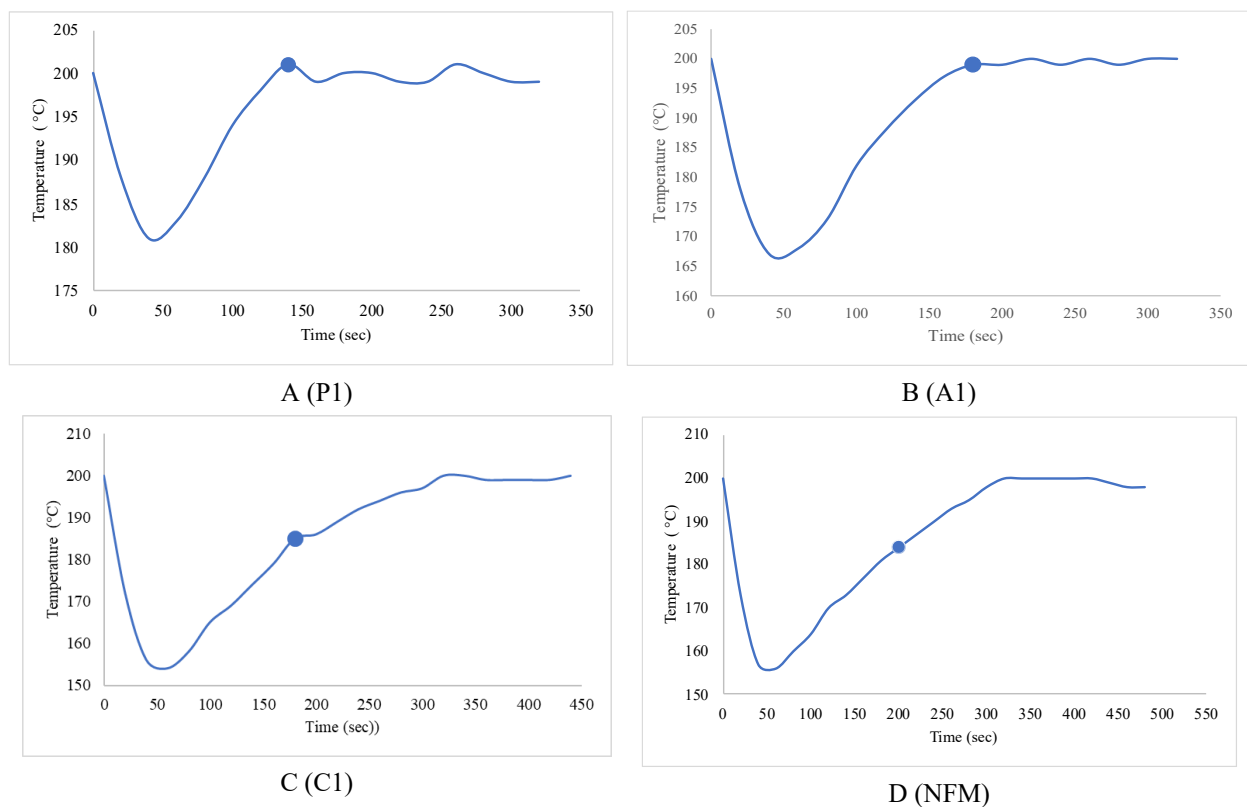


Figure 7 Temperature curves for medium roasting of coffee beans: (A) P1, (B) A1, (C) C1, and (D) NFM. The graphs show how temperature changes over time during roasting, emphasizing the variations between fermented and unfermented beans.

As shown in **Figure 7**, at approximately 50 - 100 °C, beans undergo an endothermic phase, absorbing heat and initiating browning reactions such as Maillard and Strecker reactions, leading to melanoidin formation. Around 150 °C, the process transitions to an exothermic phase, characterized by the release of water vapor, CO₂, and CO, accompanied by bean expansion. At temperatures between 180 - 200 °C, endosperm rupture triggers the first crack, accompanied by bluish smoke, aroma release, and intensified caramelization [53]. In this study, first crack during medium roasting occurred between the 2nd and 4th minute, depending on bean moisture content, with higher moisture delaying crack onset. The observed temperature range for first crack (184 - 200 °C) aligns well with previously reported values [53].

Bioactive compound profile of Liberica coffee based on LC-MS analysis

LC-MS analysis revealed a total of 57 compounds across all Liberica coffee samples, with retention times ranging from 1.85 to 17.47 min (**Table 3**). Polar compounds dominated the early region of the chromatogram, which is consistent with the use of ethanol as a polar extraction solvent. Among the fermented samples, C1 (fermented with *Saccharomyces cerevisiae*, medium roast) exhibited the highest number of detected compounds (18 compounds), followed by A1 (15 compounds), P1 (15 compounds), and the non-fermented medium-roast sample (NFM) with only 8 compounds. These results indicate that microbial fermentation enhanced chemical diversity in Liberica coffee compared with non-fermented control.

Table 3 Bioactive compounds in fermented (P1, A1, C1) and unfermented (NFM) Liberica coffee as identified using LC-MS analysis. The information provided includes retention times (RT), mass-to-charge ratio (m/z), compound names, chemical classes, and whether or not they were present (+/-).

No	RT (min)	Measured (m/z)	Calculated (m/z)	Name	Groups	P1	A1	C1	NFM
1	3.85	138.0566	138.0555	Trigoneline	Alkaloids	+	+	+	+
2	3.43	151.0369	151.0395	4-Formylbenzoic acid	Aldehydes	-	-	+	-
3	4.99	126.0564	126.0555	N-ethylmaleimide	Maleimides	+	-	+	+
4	6.97	199.1094	199.1083	Metharbital	Barbiturates	+	+	+	+
5	7.48	199.1094	163.9395	Umbelliferone	Phenolics	+	+	+	+
6	7.83	355.1032	355.1029	Chlorogenic acid	Phenolics	+	+	+	+
7	8.14	195.0890	195.0882	Caffeine	Alkaloids	+	+	+	+
8	9.04	177.0563	177.0552	Herniarin	Aromatic compounds	+	+	+	-
9	7.81	164.0439	164.0460	7-Nitroindazole	Heterocyclic compounds	-	-	+	-
10	4.99	195.0883	195.0869	4-Nitrobenzaldehyde	Aromatic aldehydes	-	-	+	-
11	5.30	153.0559	153.0326	8-Azaguanine	Nitrogen heterocycles	-	-	+	-
12	6.09	155.0824	155.0821	(S)-Hexahydropyrrolo[1,2-a]pyrazine-1,4-dione	Cyclic peptides	-	+	+	-
13	10.17	247.1128	247.1083	N-Acetyltryptophan	Amino acid derivatives	-	-	+	-
14	11.54	122.0926	122.0970	1-Phenylethylamine	Hydrazine derivatives	-	+	+	-
15	12.20	138.0634	138.0667	Isoniazid	Carboxylic acids	-	-	+	-
16	12.97	279.2304	279.2324	alpha-Linolenic acid	Fatty amides	-	-	+	-

No	RT (min)	Measured (m/z)	Calculated (m/z)	Name	Groups	P1	A1	C1	NFM
17	14.27	324.2915	324.2903	Linoleoyl ethanolamide	Carboxylic acids	+	–	+	–
18	15.69	160.0743	1160.0722	L-Hexahydro-3-imino-1,2,4-oxadiazepine-3-carboxylic acid	Alkaloid synthesis	+	–	+	–
19	7.81	286.1892	286.1919	Mepyramine	Carboxylic acids	+	–	–	–
20	9.17	117.0598	117.0552	Levulinic acid	Tropane alkaloids	+	–	–	–
21	10.42	162.0893	162.0919	(R)-Boschniakine	Triazine derivatives	+	–	–	–
22	14.24	183.1385	183.1358	2,2,4,6-Tetramethylmelamine	Fatty acid amides	+	–	–	–
23	14.82	324.2905	324.2903	Oleoylethanolamide	Fatty acid derivatives	+	–	–	–
24	15.52	326.3064	326.3059	Pipecitine	Piperidine alkaloids	+	–	–	–
25	16.68	350.3430	350.3423	Methylalpha-D-galactopyranoside	Aromatic compounds	–	+	–	–
26	2.99	151.0358	151.0330	2-Aminobenzothiazole	Amino acid derivatives	–	+	–	–
27	4.49	195.0922	130.0504	Pyroglutamic acid	Phenolics	–	+	–	–
28	8.94	193.0515	193.0501	Scopoletin	Amino acids	–	+	–	–
29	10.07	166.0888	166.0886	phenylalanine	Aromatic compounds	–	+	–	–
30	10.60	175.0849	175.0849	Indole-3-acetamide	Phenolics	–	+	–	–
31	12.90	127.0442	127.0442	1,2,3-Trihydroxybenzene	Terpenoids	–	+	–	–
32	3.16	137.1304	137.1330	Terpinolene	Heterocyclic compounds	–	–	–	+
33	5.06	196.0868	196.0875	6-hydroxymethyl-7,8-dihydropterin	Aromatic amines	–	–	–	+
34	10.13	122.0978	122.0970	2-Ethylaniline	Alkaloids	–	–	–	+

Across all samples, caffeine was the dominant compound, exhibiting the highest peak intensity with retention times of approximately 6.73 min (P1), 8.38 min (A1), 8.14 min (C1), and 12.28 min (NFM) and an m/z value of 195.0882. In addition to caffeine, several well-known coffee bioactive compounds were detected, including trigonelline, chlorogenic acid, umbelliferone, and pyroglutamic acid. Five major compounds—caffeine, trigonelline, chlorogenic acid, umbelliferone, and metharbital—were consistently detected in all samples, indicating their relative stability throughout fermentation and roasting. Caffeine remained abundant in all treatments due to the high thermal stability of 1,3,7-trimethylxanthine during roasting [54]. In contrast, trigonelline is partially degraded during roasting to form pyridine derivatives, which contribute to nutty and

roasted aroma characteristics [55]. Chlorogenic acid, the most abundant phenolic acid in coffee, undergoes thermal degradation to caffeic and quinic acids, resulting in decreasing concentrations with increasing roasting intensity [54,56]. Coumarin derivatives such as umbelliferone and herniarin are known to contribute to pleasant aromatic notes, while pyroglutamic acid and terpinolene are associated with characteristic coffee aromas. The presence of these compounds supports the role of fermentation and roasting in shaping the sensory complexity of Liberica coffee.

The Venn diagram (**Figure 8**) illustrates that although the five major compounds were shared among all samples, each fermentation treatment produced a distinct set of unique compounds. A1 contained eight unique compounds, P1 contained six, C1 contained

eight, and NFM contained only three. This pattern demonstrates that fermentation with different microorganisms can generate new secondary metabolites or modify existing compounds, thereby producing characteristic chemical fingerprints for each treatment [57]. Importantly, the increased diversity of bioactive compounds in fermented samples may also have functional and sensory implications. Phenolic compounds such as chlorogenic acid and flavonoid derivatives are known for their antioxidant activity, which plays a role in mitigating oxidative stress associated with free radical-related diseases. In addition, these compounds influence coffee flavor, where chlorogenic acids contribute to acidity and bitterness, while their degradation products enhance aroma complexity. Caffeine also contributes to bitterness and overall flavor intensity. Therefore, controlled fermentation not only enhances the diversity of

bioactive compounds but also contributes to improving both the antioxidant potential and flavor quality of Liberica coffee.

Despite its high sensitivity and broad detection capability, LC-MS analysis has several limitations when applied to complex matrices such as coffee. The presence of numerous structurally similar compounds may lead to co-elution or overlapping signals, which can affect compound identification and quantification accuracy. In addition, ion suppression or enhancement effects may occur due to matrix complexity, potentially influencing signal intensity. Variability in processing conditions, including roasting temperature, fermentation time, and microbial activity, may further alter compound composition and contribute to differences in analytical outcomes. Therefore, the results of this study should be interpreted with consideration of these analytical and process-related limitations.

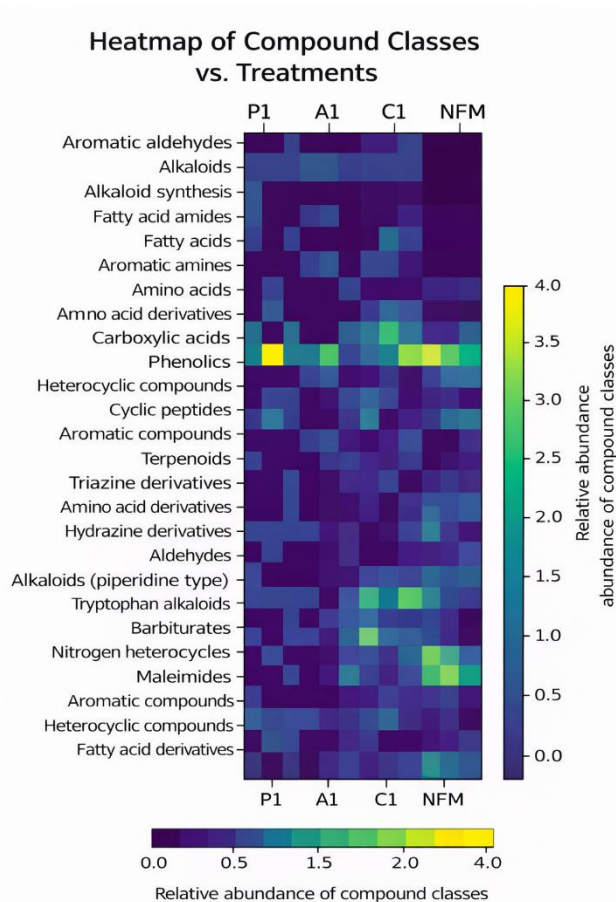


Figure 8 Heatmap of compound classes versus treatments (P1, A1, C1, and NFM). Color intensity represents the relative abundance of each compound class, with darker colors indicating lower abundance and brighter colors indicating higher abundance. The heatmap illustrates differences in chemical composition among fermented and non-fermented coffee samples.

Antioxidant activity

DPPH assay results indicate that all Liberica coffee extracts, both fermented and non-fermented, exhibited strong to very strong antioxidant activity ($IC_{50} < 100$ ppm). The lowest IC_{50} values were observed in samples fermented with *Saccharomyces cerevisiae* and *Lactobacillus plantarum*, indicating higher radical scavenging capacity compared to non-fermented coffee (Table 4). One-Way ANOVA analysis showed no significant differences among samples and the positive control ($p > 0.05$), suggesting that fermented Liberica coffee possesses antioxidant potential comparable to ascorbic acid [58]. The enhanced antioxidant activity in fermented coffee is associated with microbial enzymatic actions, such as β -glucosidase, esterase, and phenolic oxidoreductase, which hydrolyze complex compounds into free phenolics, thereby increasing the availability of bioactive components such as chlorogenic acid and ferulic acid [41,59]. These findings are consistent with

previous studies reporting significant reductions in IC_{50} values following fermentation with lactic acid bacteria and yeast, where antioxidant activity increased from strong to very strong categories [60,61].

Fermentation using *Aspergillus* also improved antioxidant activity compared to non-fermented coffee, although to a lesser extent than bacterial and yeast fermentation. This improvement is attributed to the ability of *Aspergillus* enzymes to hydrolyze bound phenolic compounds into simpler, soluble forms, thus enhancing antioxidant capacity [62]. In addition, roasting degree affected antioxidant stability; medium roasting preserved higher antioxidant activity than dark roasting due to thermal degradation and polymerization of phenolic compounds at higher temperatures [63,64]. Overall, microbial fermentation combined with appropriate roasting conditions plays a crucial role in enhancing and maintaining the antioxidant activity of Liberica coffee.

Table 4 Antioxidant activity of fermented coffee samples based on IC_{50} values and activity categories. This table compares the antioxidant capacity of each treatment group, including the control.

Sample	$IC_{50} \pm SD$ (ppm)	Activity
Ascorbic acids	$11.04^a \pm 0.00$	Very strong
P1	$35.42^b \pm 3.566$	Very strong
P2	$41.28^b \pm 0.601$	Very strong
C1	$33.16^b \pm 0.097$	Very strong
C2	$66.24^c \pm 0.553$	Strong
A1	$68.58^c \pm 0.565$	Strong
A2	$78.97^c \pm 0.488$	Strong
NFM	$77.24^c \pm 0.552$	Strong
NFD	$41.94^b \pm 0.000$	Very strong

Relationship between PCA, compound classes, and antioxidant activity (IC_{50})

PCA based on the relative intensities of compound classes showed a clear separation between fermented coffee samples (P1, A1, C1) and the non-fermented sample (NFM). The first two principal components (PC1 and PC2) explained the largest proportion of variation in chemical composition among samples. P1 and C1 clustered within the same quadrant, indicating similar metabolite profiles, whereas A1 occupied a

distinct position, suggesting a slightly different chemical character. NFM was located farthest from the fermented samples, confirming that fermentation plays a major role in modulating the bioactive compound composition of Liberica coffee.

The compound-class heatmap further demonstrated that fermentation increased the relative abundance of several major classes, particularly phenolics, carboxylic acids, alkaloids, nitrogen heterocycles, and amino acid derivatives. Phenolic and

carboxylic acid classes were dominant in P1 and C1, whereas A1 exhibited relatively higher levels of alkaloids, nitrogen heterocycles, and barbiturates. In contrast, the NFM sample showed lower relative abundances for most of these compound classes.

When correlated with IC₅₀ values (Table 5), samples with higher abundances of phenolics and carboxylic acids (P1 and C1) exhibited the strongest antioxidant activity, as indicated by their lower IC₅₀ values (35.42 ± 3.566 ppm for P1 and 33.16 ± 0.097 ppm for C1). Conversely, A1 and NFM, which showed lower relative intensities of phenolic compounds, had higher

IC₅₀ values (68.58 ± 0.565 ppm and 77.24 ± 0.552 ppm, respectively). Overall, PCA and heatmap analyses confirm a negative correlation between the abundance of bioactive compound classes—particularly phenolics, alkaloids, and nitrogen heterocycles—and IC₅₀ values. Higher abundance of these compounds is associated with stronger antioxidant activity of coffee extracts (Figure 9). These results demonstrate that controlled fermentation, especially using *Lactobacillus plantarum* and *Saccharomyces cerevisiae*, is effective in enhancing antioxidant compound content and improving the functional quality of Liberica coffee.

Table 5 Spearman rank correlation between compound classes and response variable.

Compound Class	r (Spearman)	p-value	Interpretation
Phenolics	−0.80	0.20	Strong negative (not significant)
Carboxylic acids	−0.80	0.20	Strong negative (not significant)
Alkaloids	−0.20	0.80	Weak correlation
Nitrogen heterocycles	−0.40	0.60	Moderate (not significant)
Amino acid derivatives	−0.40	0.60	Moderate (not significant)

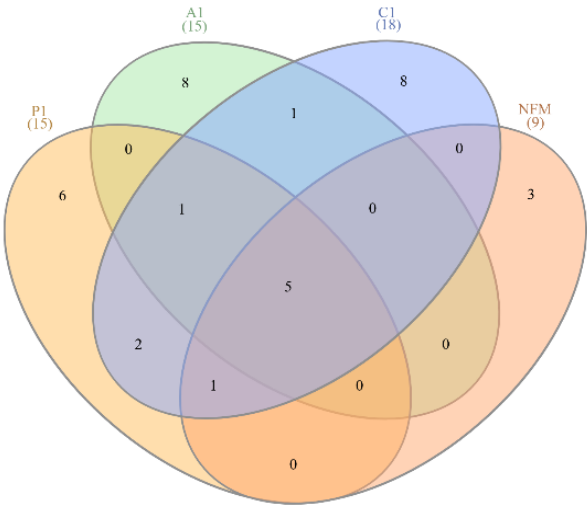


Figure 9 Venn diagram showing the distribution and overlap of bioactive compounds among coffee samples P1, A1, C1, and NFM. The numbers indicate unique and shared compounds detected in each sample, illustrating similarities and differences in chemical composition between fermented and non-fermented coffees.

Sensory evaluation

Based on cupping results, coffee with a medium roasting level was more preferred by panelists than dark-roasted coffee. At the medium roast level, aroma intensity was stronger, bean color was darker, natural

sugars began to caramelize, and the flavor was slightly sweet with a well-balanced combination of taste, aroma, and acidity [65]. Fermentation using three different microorganisms produced volatile and non-volatile compounds that influenced the flavor and aroma

characteristics of coffee. Overall, coffee fermented with *A. niger*. and roasted at a medium level exhibited the highest sensory scores compared with non-fermented coffee.

The organoleptic test showed that fermentation using different microorganisms—*Lactobacillus plantarum*, *Aspergillus*, and *Saccharomyces cerevisiae*—resulted in varying effects on sensory quality, mainly influenced by microbial type and roasting degree. Fermentation with *L. plantarum* at medium roast (P1) produced a significant improvement in quality, with a total score of 78.92, higher than that of non-fermented coffee (NFM, 72.5). The improvement was particularly evident in uniformity, indicating the formation of volatile and non-volatile compounds that enriched coffee flavor and aroma (Table 5). This is consistent with reports that *L. plantarum* can degrade pectin and glucose into lactic acid, while green coffee beans naturally contain various organic acids contributing to aroma and flavor [66]. Previous studies [28] also reported that lactic acid fermentation enhances the formation of volatile compounds such as esters, which impart caramel, fruity, and exotic notes to coffee (Table 6).

In contrast, at dark roast (P2), the difference between fermented and non-fermented samples was relatively small (73.42 vs. 72.5), suggesting that high roasting temperatures degrade fermentation-derived aromatic compounds. Fermentation using *Aspergillus* at medium roast (A1) also improved sensory quality (79.25), particularly in fragrance, flavor, and aftertaste. This microorganism produces proteolytic enzymes such as acid protease, glutaminase, and aspartase, which increase amino acid content and aroma precursors. However, at dark roast (A2), the benefits of fermentation decreased due to thermal degradation of volatile

compounds, in agreement with reports describing caramel-like aroma formation through pyrazines, thiols, and furanones [67].

Fermentation with *S. cerevisiae* at medium roast (C1) increased the total score (75) compared with non-fermented coffee, although not all sensory attributes were equally improved. At dark roast (C2), the total score decreased (69.45), presumably due to volatilization of aroma compounds during roasting, as indicated by the very low moisture content (0.0366%). Fermentation can hydrolyze carbohydrates into reducing sugars, generating organic acids and decreasing caffeine—one of the main contributors to bitterness. This enzymatic activity promotes fruity, floral, and spicy characteristics [68,69].

Overall, fermentation modifies the chemical composition of coffee beans through the formation of organic acids, alcohols, and esters that strongly influence sensory quality. However, fermentation is most effective at the medium roasting level, while high temperatures during dark roasting tend to degrade aromatic compounds. These findings emphasize that fermentation success is strongly dependent on microbial type, fermentation conditions, and roasting parameters. Microbial activity during fermentation modifies chemical compounds in coffee beans, including organic acids, alcohols, and esters, which directly affect final sensory quality. Microbial type, fermentation duration, and fermentation medium play crucial roles in determining the success of the process. Therefore, comparative analysis of sensory values among different coffee species (*Liberica*, *Arabica*, and *Robusta*) fermented with microorganisms such as *L. plantarum* and *S. cerevisiae* provides important insight into the effectiveness of fermentation in improving coffee quality.

Table 6 Sensory attributes of fermented coffee samples subjected to different roasting treatments. The table gives information on the aromas and flavors of the fermented coffee samples from various treatment groups.

Parameter	Sample							
	P1	P2	A1	A2	C1	C2	NFM	NFD
Fragrance	7.33 ± 0.3	6.83 ± 0.2	7.58 ± 0.3	7.00 ± 0.0	7.08 ± 0.1	6.33 ± 0.1	7.5 ± 0.0	7.5 ± 0
Flavor	7.67 ± 0.1	7.17 ± 0.1	7.67 ± 0.1	6.92 ± 0.3	7.0 ± 0.0	6.42 ± 0.1	7.25 ± 0.1	7.5 ± 0
Aftertaste	7.33 ± 0.3	6.92 ± 0.3	7.50 ± 0.1	7 ± 0.5	7.0 ± 0.2	6.08 ± 0.1	7.25 ± 0.1	7.5 ± 0.01

Parameter	Sample							
	P1	P2	A1	A2	C1	C2	NFM	NFD
Salt/Acid	7.17 ± 0.1	6.83 ± 0.2	7.50 ± 0.0	6.58 ± 0.7	6.83 ± 0.2	6.0 ± 0.0	7.25 ± 0.1	7.25 ± 0.1
Bitter/sweet	7.08 ± 0.1	6.83 ± 0.1	7.50 ± 0.0	6.42 ± 0.1	6.5 ± 0.2	6.08 ± 0.1	7.25 ± 0.1	7.75 ± 0.25
Mouthfeel	7.42 ± 0.3	7.08 ± 0.1	7.17 ± 0.1	7.08 ± 0.1	6.75 ± 0.2	6.0 ± 0.0	7.25 ± 0.1	7.25 ± 0.1
Uniformity	10 ± 0.00	10 ± 0.0	10 ± 0.0	10.00 ± 0	10 ± 0.0	10 ± 0.0	10 ± 0.1	10 ± 0.01
Balance	7.33 ± 0.1	6.92 ± 0.3	7.25 ± 0.1	6.67 ± 0.5	6.92 ± 0.3	6.33 ± 0.2	7.25 ± 0.1	7.25 ± 0.1
Clean Cup	10 ± 0.00	10 ± 0.00	10 ± 0.0	10.00 ± 0	10 ± 0.0	10 ± 0.0	10.0 ± 0.0	10.0 ± 0.01
Overall	7.58 ± 0.1	6.83 ± 0.2	7.08 ± 1.4	6.83 ± 0.2	6.92 ± 0.1	6.17 ± 0.1	7.25 ± 0	7.5 ± 0.01
Total Score	78.92 ± 1.3	73.42 ± 1.6	79.25 ± 1.3	74.5 ± 2.4	75 ± 1.3	69.45 ± 0.8	72.5 ± 0.1	72.5 ± 0.1

Sample	Roasting Medium		Roasting Dark	
	Aroma	Flavor (Taste)	Aroma	Flavor (Taste)
<i>Lactobacillus plantarum</i>	Peanut, spices; sweet like ripe banana/berries	Slightly dry, yet smooth and pleasant on the throat	Palm sugar, nuts; intense palm sugar, chocolate	Full body, sweet with a bitter finish; low acidity masked by sweetness and bitterness
<i>Aspergillus niger</i>	Roasted almond, spices; ripe banana and palm sugar	Ripe orange, sugarcane juice, slight nectar; light and sweet mouthfeel; low acidity	Dark chocolate and roasted caramel; intensely sweet chocolate	Chocolate, caramel, palm sugar with a bitter finish; full body, slightly sticky mouthfeel; low acidity
<i>Saccharomyces cerevisiae</i>	Ripe fruit, sweet nuts; palm sugar and caramel	Caramel, chocolate, slight honey; textured mouthfeel, sweet with a bitter finish; low acidity.	Roasted nuts, cocoa; unripe fruit and intense chocolate	Peanut, green vegetables, slight citrus; dry mouthfeel, bitter finish; moderate and slightly sharp acidity

Proximate analysis

Proximate analysis (Table 7) showed that 24 h controlled fermentation led to measurable changes in the composition of Liberica coffee, reflecting microbial activity and its impact on product quality and stability. Moisture content varied depending on the microorganism and processing conditions. Fermented samples generally exhibited higher moisture during wet processing but were effectively reduced after roasting, with all treatments meeting the SNI 8964:2021 limit (< 5%). This indicates good storage stability and a low risk of microbial spoilage. However, excessive moisture loss during roasting may negatively affect the retention of volatile aroma compounds. Proximate analysis included determination of moisture, ash, protein, fat, crude fiber, and total carbohydrate contents (Table 7).

This analysis provides quantitative information on macronutrient composition, which is essential for assessing coffee quality, safety, and nutritional value. In addition, the results can be used as a basis for product quality standardization, formulation development, and studies on the effects of processing on the chemical composition of coffee beans and powder.

Moisture content of fermented coffee was influenced by microbial type and fermentation method. Fermentation with *S. cerevisiae* resulted in lower moisture content, whereas fermentation with *A. niger* and *L. plantarum* increased moisture due to wet fermentation, which allows beans to absorb water [70]. Roasting also reduced moisture content; higher temperature and longer roasting time led to greater evaporation [71], with approximately 8% loss at

medium roast and up to 20% at dark roast. All treatments met the maximum moisture limit of < 5% according to SNI 8964:2021, indicating good storage stability and low risk of mold growth. However, excessively low moisture content may reduce the stability of aromatic compounds during high-temperature roasting.

Fermentation increased protein content compared with non-fermented coffee, especially at medium roast. This increase is attributed to microbial activity that breaks down macromolecules into simpler compounds, making protein more available. *Lactobacillus plantarum* produced the highest protein content through protease activity and essential amino acid synthesis, particularly at 12 h fermentation, which represents optimal growth time [72,73]. *S. cerevisiae* and *Aspergillus* also increased protein content through extracellular enzyme secretion and single-cell protein formation, although to a lesser extent [74].

Ash content of all samples complied with SNI 8964-2021 requirements (maximum 5%). Fermented coffee generally exhibited lower ash content, indicating that fermentation reduced organic compounds that would otherwise form ash after combustion. Coffee contains minerals such as potassium, calcium, magnesium, phosphorus, and sulfur [60]. Some dark-roasted samples showed relatively higher ash content, consistent with reports that roasting reduces organic

fractions, thereby increasing the relative proportion of inorganic material [75].

Fermentation affected fat content compared with non-fermented coffee, which tended to remain close to the natural composition of coffee beans. Dark-roasted coffee exhibited higher fat content than medium-roasted coffee due to moisture loss [76]. Coffee lipids, concentrated in the wax and oil layers of beans, play an important role in flavor development and contain various fatty acids and derivatives of 5-hydroxytryptamides [77]. Arabica coffee contains 11.04% - 13.22% fat, whereas Robusta contains 6.68% - 16.67%. Fermentation with *Lactobacillus plantarum*, *Aspergillus*, and *Saccharomyces cerevisiae* produced variable fat contents, indicating that microbial type and fermentation conditions determine lipid changes.

The decrease in carbohydrate content of fermented coffee is related to microbial utilization of carbohydrates as an energy source [78]. Microorganisms such as *Lactobacillus plantarum*, *Saccharomyces cerevisiae*, and *Aspergillus* hydrolyze polysaccharides and oligosaccharides into simple sugars, which are subsequently converted into metabolites such as organic acids, ethanol, and volatile compounds that influence coffee flavor and aroma. In contrast, non-fermented coffee does not undergo microbial carbohydrate degradation, resulting in relatively higher and more stable carbohydrate content.

Table 7 Proximate composition of fermented coffee samples. The table contains information on the proximate composition of the samples from various treatment groups.

Parameters	Samples							
	P1	A1	C1	NFM	P2	A2	C2	NFD
Moisture (%)	1.3514	1.0039	0.4760	0.7497	1.1894	0.4624	0.0366	1.2296
Protein Content (%)	21.951	19.3012	21.9332	18.4546	21.9259	18.4055	17.5758	17.5407
Ash Content (%)	3.9265	3.4143	3.3275	4.8153	4.0985	3.5456	3.4655	4.9839
Lipids Content (%)	12.9954	12.6786	12.3400	11.5975	14.0407	14.7317	15.2080	12.1320
Carbohydrate Content (%)	59.7716	64.4977	61.9233	64.3829	58.7455	61.9591	63.7141	64.1138

Conclusions

Fermentation of Liberica coffee in a bioreactor showed controlled trends in pH reduction and temperature profiles, indicating active microbial metabolism under monitored conditions. Gas production

data (CO₂, H₂, and CH₄) provided additional indications of microbial activity; however, these observations should be interpreted with caution and require further validation for quantitative assessment. The fermentation process significantly improved coffee sensory quality,

with fermented samples subjected to medium roasting exhibiting higher sensory scores than non-fermented coffee. The best overall performance was observed in sample A1, which fell into the “very good” category. LC-MS analysis demonstrated that fermentation increased the diversity of bioactive compounds compared with non-fermented coffee, with caffeine remaining the dominant compound in all samples. The highest number of bioactive compounds (18 compounds) was detected in sample C1. Furthermore, fermented Liberica coffee showed higher antioxidant activity, particularly in samples C1 and P1, which exhibited the greatest antioxidant capacity.

Importantly, among the tested microorganisms, *Aspergillus niger* produced the best sensory performance, *Saccharomyces cerevisiae* resulted in the highest diversity of bioactive compounds, and both *S. cerevisiae* and *Lactobacillus plantarum* showed the strongest antioxidant activity. These findings highlight the distinct functional roles of each microorganism and provide a basis for selecting targeted fermentation strategies depending on desired coffee quality attributes. Overall, controlled microbial fermentation represents a promising strategy to enhance the sensory quality, chemical diversity, and functional properties of Liberica coffee, with potential applications in specialty coffee processing and value-added product development. Future studies should focus on optimizing fermentation parameters and scaling up the bioreactor system to improve industrial applicability and process efficiency.

Acknowledgements

The authors would like to express their sincere gratitude to the University Centre of Excellence E2-KOLIM for its support and to the Faculty of Science and Technology for providing laboratory facilities that enabled this research. The author gratefully acknowledges financial support from the Directorate of Research, Technology, and Community Services (DRTPM), Ministry of Higher Education, Science, and Technology of the Republic of Indonesia (2025). This work was supported under Grant Number 071/C3/DT.05.00/PL/2025 and Contract Number 29/UN21.11/PT.01.05/SPK/2025.

Conflicts of Interest

The authors declare that they have no known competing financial interests or personal relationships

that could have appeared to influence the work reported in this paper

Declaration of generative AI in scientific writing

The authors acknowledge the use of generative AI tools (e.g., QuillBot, ChatGPT by OpenAI, Grammarly, and Mendeley) in the preparation of this manuscript, specifically for language editing and grammatical refinement. These tools were utilized solely to improve clarity and readability. No AI was used for content generation, data analysis, or interpretation. The authors assume full responsibility for the integrity, accuracy, and conclusions presented in this work.

CRediT author statement

Indra Lasmana Tarigan: Conceptualization; Methodology; Software; Formal analysis; Investigation; Writing-Original Draft; Writing-Review and Editing. **Amanda Fitri Rahmawati:** Methodology; Software; Formal analysis; Investigation; Writing-Original Draft. **Salmiyah Oktrariyani:** Investigation and Software. **Madyawati Latief:** Conceptualization; Methodology; Writing-Original Draft. **Noor Ariefandie Febrianto:** Data curation; Writing-Review and Editing, Supervision. **Sutrisno Sutrisno:** Conceptualization, Data curation; Writing-Review and Editing, Supervision, and Funding acquisition.

Data Availability Statement

The data presented in this study are included in this article. Additional raw data are available from the corresponding author upon reasonable request.

References

- [1] SA Halim-Lim, WAAQI Wan-Mohtar, S Surapinchai and NAZ Azizan. Optimum condition of roasting process of Liberica coffee towards the local and international preference. *Food Research* 2022; **6(3)**, 115-123.
- [2] D Hanifah, D Herawati and N Andarwulan. Karakteristik fisikokimia dan kapasitas antioksidan kopi Liberika dari Kabupaten Tanjung Jabung Barat, Jambi. *Jurnal Teknologi dan Industri Pangan* 2022; **33(1)**, 39-51.
- [3] NA Wibowo, W Mangunwardoyo, TJ Santoso and Yasman. Investigation of volatile compounds of Liberica coffee beans fermented at varying degrees of roasting. *Food Research* 2024; **8(3)**, 43-

- 53.
- [4] RA Fadri, K Sayuti, N Nazir and I Suliansyah. The effect of temperature and roasting duration on physical characteristics and sensory quality of singgalang arabica coffee (*Coffea arabica*) agam regency. *Journal of Applied Agricultural Science and Technology* 2019; **3(2)**, 189-201.
- [5] LJC Ferreira, M de Souza Gomes, LM de Oliveira and LD Santos. Coffee fermentation process: A review. *Food Research International* 2023; **169**, 112793.
- [6] H Wu, CG Viejo, S Fuentes, FR Dunshea and HAR Suleria. Evaluation of spontaneous fermentation impact on the physicochemical properties and sensory profile of green and roasted arabica coffee by digital technologies. *Food Research International* 2024; **176**, 113800.
- [7] GI Puerta Quintero. Development of controlled fermentation processes to add value to coffee quality. In: ASIC 25th International Conference on Coffee Science, Colombian. 2014, p. 42-45.
- [8] F Palladino, PRF Marcelino, AE Schlogl, ÁHM José, R de Cássia Lacerda Brambilla Rodrigues, DL Fabrino, IJB Santos and CA Rosa. Bioreactors: Applications and innovations for a sustainable and healthy future: A critical review. *Applied Sciences* 2024; **14(20)**, 9346.
- [9] AK Ilham, IL Tarigan, II Ramadhan, MF Afrianto, M Latief, Sutrisno. Preliminary Data on Monitoring the Fermentation of Liberica Coffee using a Sensor-Integrated Mini Bioreactor. *Coffee Science*. 2026; 21,e212420. 10.25186/.v21i.2420
- [10] MC da Silva, E da Silva G de Castro, J do N Barreto, PV de Oliveira Martins, GL da Silva, RF da Silva, DG dos Santo, O Freitas-Silva, JBP Simão, AI da Silva Junior and ADP Netto. Ochratoxin a levels in fermented specialty coffees from Caparaó, Brazil: Is it a cause of concern for coffee drinkers?. *Food Additives and Contaminants - Part A* 2021; **38(11)**, 1948-1957.
- [11] T Feng and J Wang. Oxidative stress tolerance and antioxidant capacity of lactic acid bacteria as probiotic: A systematic review. *Gut Microbes* 2020; **12(1)**, 1801944.
- [12] H Izzati, J Jalaluddin, Z Ginting, E Kurniawan and S Sulhatun. Pengaruh waktu fermentasi terhadap mutu kopi menggunakan bakteri asam laktat dari yakult. *Chemical Engineering Journal Storage* 2022; **2(3)**, 61-74.
- [13] M Haile and WH Kang. The role of microbes in coffee fermentation and their impact on coffee quality. *Journal of Food Quality* 2019; **2019(1)**, 4836709.
- [14] SA Setianingsih, EK Sari and MK Putri. Pengaruh derajat penyangraian terhadap kadar asam klorogenat kopi robusta temanggung dengan metode spektrofotometri UV-Vis. *Jurnal Jamu Kusuma* 2023; **3(1)**, 7-14.
- [15] T Dajoh, RA Bara, E Angkouw, M Ompi, RAJ Lintang and C Lumenta. Uji aktivitas antibakteri dan Anti-Uv *Phyllidiella nigra* dan bakteri simbiotiknya dari perairan tanjung mandolang. *Jurnal Pesisir Dan Laut Tropis* 2020; **8(2)**, 61-71.
- [16] TW Imanisa, E Mardawati, N Masruchin. Produksi enzim xilanase dari *Aspergillus niger* melalui metode fermentasi terendam dalam valorisasi tandan kosong kelapa sawit sebagai produk biorefineri bernilai tambah. *Biomass, Biorefinery, and Bioeconomy* 2023; **1(1)**, 14-19.
- [17] N Sa'aid, JS Tan, MS Mohamed and L Muthulakshmi. Comparative analysis of Lactobacillus spp. fermentation in five fruit drinks: Impacts on lactic acid production and cell viability. *Malaysian Applied Biology* 2025; **54(2)**, 55-64.
- [18] T Purwoko, Suranto, R Setyaningsih, SD Marliyana. Chlorogenic Acid and Caffeine Content of Fermented Robusta Bean. *Biodiversitas Journal of Biological Diversity*. 2022. **23(2)**, 902-906.
- [19] IL Tarigan, Z Adriliana Z, AA Putri, NA Febrianto, M Latief and Sutrisno. Changes in the chemical compound and sensory profiles of Liberica fermentation (*Coffea liberica*) with cellulolytic bacteria *Alcaligenes sp* and *Exiguobacterium indicum*. *Coffee Science* 2024; **19**, 192258.
- [20] HA Tanauma, G Citraningtyas, WA Lolo. Aktivitas antibakteri ekstrak biji kopi robusta (*Coffea canephora*) terhadap bakteri *Escherichia coli*. *Pharmakon* 2016; **5(4)**, 243-251.
- [21] S Baliyan, R Mukherjee, A Priyadarshini, A Vibhuti, A Gupta, RP Pandey and CM Chang. Determination of antioxidants by DPPH radical scavenging activity and quantitative phytochemical analysis of *Ficus religiosa*.

- Molecules* 2022; **27**(4), 1326.
- [22] JM Maligan, ATE Wibowo, NZ Anggono, SU Kosasih and YK Putra. Pengujian karakteristik sensori kopi robusta tirtoyudo natural. *Prosiding Seminar Nasional Instiper* 2022; **1**(1), 299-305.
- [23] AFP Cika, Y Uztamila, AS Effendy, A Syarif and I Hajar. Pengaruh pH fermentasi dan putaran pengadukan pada fermentasi molasses terhadap produksi bioetanol. *Jurnal Pendidikan dan Teknologi Indonesia* 2022; **2**(1), 41-47.
- [24] H Elhalis, J Cox and J Zhao. Ecological diversity, evolution and metabolism of microbial communities in the wet fermentation of Australian coffee beans. *International Journal of Food Microbiology* 2020; **321**, 108544.
- [25] S Avallone, B Guyot, JM Brillouet, E Olguin and JP Guiraud. Microbiological and biochemical study of coffee fermentation. *Current Microbiology* 2001; **42**, 252-256.
- [26] J Suryadi, AR Subagja, AN Amalia and T Hariyadi. The effect of temperature and fermentation time using local microorganism on robusta coffee characteristics. *FLUIDA* 2025; **18**(1), 1-8.
- [27] LL Pereira and TR Moreira. *Quality Determinants in Coffee Production*. Springer International Publishing, Germany, 2021, p. 303-308.
- [28] SR Evangelista, CF Silva, MGP da Cruz Miguel, C de Souza Cordeiro, ACM Pinheiro, WF Duarte and RF Schwan. Improvement of coffee beverage quality by using selected yeasts strains during the fermentation in dry process. *Food Research International* 2014; **61**, 183-195.
- [29] B Zhang, M Gebrekidan, Z Yan, X Yi and J Bao. Very high thermotolerance of an adaptive evolved *Saccharomyces cerevisiae* in cellulosic ethanol fermentation. *Fermentation* 2023; **9**(4), 393
- [30] BP Khatri, T Bhattarai, S Shrestha and J Maharjan. Alkaline thermostable pectinase enzyme from *Aspergillus niger* strain MCAS2 isolated from Manaslu Conservation Area , Gorkha , Nepal. *SpringerPlus* 2015; **4**, 488.
- [31] DC Aryani, HMW den Besten and MH Zwietering. Quantifying variability in growth and thermal inactivation kinetics of *Lactobacillus plantarum*. *Applied and Environmental Microbiology* 2016; **82**(16), 4896-4908.
- [32] P Popova-krumova, S Danova, N Atanasova and D Yankov. Lactic acid production by *Lactiplantibacillus plantarum* AC 11S—kinetics and modeling. *Microorganisms* 2024; **12**(4), 739.
- [33] MI Tulasi, NDFK Foeh and AIR Detha. Studi literatur senyawa metabolit bakteri asam laktat dan kegunaannya dalam mengoptimisasi kesehatan hewan. *Jurnal Veteriner Nusantara* 2024; **7**(2), 262-274.
- [34] GM Walker and GG Stewart. *Saccharomyces cerevisiae* in the production of fermented beverages. *Beverages* 2016; **2**(4), 30.
- [35] W Sun, MH Shahrajabian and M Lin. Research progress of fermented functional foods and protein factory-microbial fermentation technology. *Fermentation* 2022; **8**(12), 688.
- [36] HM Lisboa, MB Pasquali, AI dos Anjos, AM Sarinho, ED de Melo, R Andrade, L Batista, J Lima, Y Diniz and A Barros. Innovative and sustainable food preservation techniques: Enhancing food quality, safety, and environmental sustainability. *Sustainability* 2024; **16**(18), 8223.
- [37] B Hariyanto, F Fanani and SE Nugroho. Rekayasa fermentasi kopi an aerobik dengan metode karbonik dan semi karbonik maserasi. *Jurnal Pengembangan Potensi Laboratorium* 2022; **1**(2), 79-85.
- [38] C Liu, W Dong, Y Yang, W Zhao, W Zeng, Y Litti, C Liu and B Yan. The effect of - CO₂ sparging on high - solid acidogenic fermentation of food waste. *Waste Disposal & Sustainable Energy* 2025; **7**, 27-39.
- [39] M Guadalupe-daqui, RM Goodrich-schneider, PJ Sarnoski, JC Carriglio, CA Sims, BJ Pearson and AJ MacIntosh. The effect of CO₂ concentration on yeast fermentation: Rates, metabolic products, and yeast stress indicators. *Journal of Industrial Microbiology and Biotechnology* 2023; **50**(1), kuad001.
- [40] X Shen, Q Wang, H Wang, G Fang, Y Li, J Zhang and K Liu. Microbial characteristics and functions in coffee fermentation: A review. *Fermentation* 2025; **11**(1), 5.
- [41] P Filannino, RD Cagno and M Gobbetti. Metabolic and functional paths of lactic acid bacteria in plant foods: Get out of the labyrinth. *Current Opinion in Biotechnology* 2018; **49**, 64-72.

- [42] H Juniar. Pengaruh aspergillus niger pada pembuatan bioetanol dari limbah kulit ubi kayu dan kulit nanas. *Jurnal Redoks* 2021; **6(1)**, 52.
- [43] Y Guan, X Xu, C Liu, J Wang, C Niu, F Zheng and Q Li. Evaluating the physiology and fermentation performance of the lager yeast during very high gravity brewing with increased temperature. *LWT* 2023; **173**, 114312.
- [44] E Andrada, A Marquez, EP Chagra Dib, P Gauffin-Cano, RB Medina. Corn stover silage inoculated with ferulic acid esterase Producing *L. johnsonii*, *L. plantarum*, *L. fermentum*, and *L. brevis* Strains: Fermentative and nutritional parameters. *Fermentation* 2023; **9(4)**, 331.
- [45] TP Dianda and PH Suharti. Pengaruh waktu dan kadar etanol pada maserasi lidah buaya terhadap antiseptik hand sanitizer gel. *Jurnal Teknologi Separasi* 2022; **22(4)**, 1000-1008.
- [46] P Nassanga, G Mauriello, SO Ochaya and D Ongeng. Genera of lactic acid bacteria isolated from a maize-based fermented beverage (*Kwete*) consumed by ethnic communities in Northern Uganda. *Discover Food* 2026; **6**, 131
- [47] KM Muñoz-Páez, EL Alvarado-Michi, G Buitrón and I Valdez-Vazquez. Distinct effects of furfural, hydroxymethylfurfural and its mixtures on dark fermentation hydrogen production and microbial structure of a mixed culture. *International Journal of Hydrogen Energy* 2019; **44(4)**, 2289-2297.
- [48] HM Poggi-Varaldo, KM Munoz-Paez, C Escamilla-Alvarado, PN Robledo-Narváez, MT Ponce-Noyola, G Calva-Calva, E Ríos-Leal, J Galíndez-Mayer, C Estrada-Vázquez, A Ortega-Clemente and NF Rinderknecht-Seijas. Biohydrogen, biomethane and bioelectricity as crucial components of biorefinery of organic wastes: A review. *Waste Management & Research: The Journal for a Sustainable Circular Economy* 2014; **32(5)**, 353-365.
- [49] ECH Chua, SK Wee, J Kansedo, SY Lau, KH Lim, SS Dol and AN Lipton. Biological hydrogen energy production by novel strains *Bacillus paramycoides* and *Cereibacter azotoformans* through dark and photo fermentation. *Energies* 2023; **16(9)**, 3807.
- [50] MAA Rodrigues, MLA Borges, AS Franca, LS Oliveira and PC Corrêa. Evaluation of physical properties of coffee during roasting. *Agricultural Engineering International: the CIGR Journal of Scientific Research and Development* 2002; **4**, 026030.
- [51] WB Sunarharum, DJ Williams and HE Smyth. Complexity of coffee flavor: A compositional and sensory perspective. *Food Research International* 2014; **62**, 315-325.
- [52] N Yergenson and DE Aston. Monitoring coffee roasting cracks and predicting with in situ near-infrared spectroscopy. *Journal of Food Process Engineering* 2019; **43(2)**, 13305.
- [53] H Siebald, M Moller, F Lenz, S Kirchner and O Hensel. Acoustic condition monitoring of coffee beans, during the roasting process. *LWT* 2024; **199**, 116119.
- [54] A Sholikhatia, BT Sukoharjantib and Y Rusidah. Potensi ekstrak kopi (*Coffea* Sp.) sebagai antioksidan: Review. *Jurnal Medika Indonesia* 2023; **4(2)**, 30-38.
- [55] N Konstantinidis, H Franke, S Schwarz and DW Lachenmeier. Risk assessment of trigonelline in coffee and coffee by-products. *Molecules* 2023; **28(8)**, 3460.
- [56] M Fuller and NZ Rao. The effect of time, roasting temperature, and grind size on caffeine and chlorogenic acid concentrations in cold brew coffee. *Scientific Reports* 2017; **7**, 17979.
- [57] I Budiman, F Wahyudi, Y Yunardi and H Meilina. Studi fermentasi biji kopi menggunakan enzim proteolitik. *Jurnal Serambi Engineering* 2021; **6(4)**, 2228-2235.
- [58] E Murlida, S Noviasari, C Nilda, S Rohaya, F Rahmi and M Muzaifa. Chemical characteristics of cascara tea from several varieties of coffee in Aceh Province. *IOP Conference Series: Earth and Environmental Science* 2021; **667**, 012078
- [59] SJ Hur, SY Lee, YC Kim, I Choi and GB Kim. Effect of fermentation on the antioxidant activity in plant-based foods. *Food Chemistry* 2014; **160**, 346-356.
- [60] M Latief, R Maharani, IL Tarigan and Sutrisno. Coffee improvement by wet fermentation using *Lactobacillus plantarum*: Sensory studies, proximate analysis, antioxidants, and chemical compounds. *Jurnal Rekayasa Kimia dan Lingkungan* 2023; **17(2)**, 134-148.
- [61] AHM Fansukri, Novelina and N Nazir. The effect of saccharomyces cerevisiae addition on the

- antioxidant activity of arabica coffee beans (*Coffea arabica*) using natural fermentation method. *AJARCDE (Asian Journal of Applied Research for Community Development and Empowerment)*. 2024; **8(2)**, 196-202.
- [62] Z Gülsünoğlu, R Purves, F Karbancioglu-Guler and M Kilic-Akyilmaz. Enhancement of phenolic antioxidants in industrial apple waste by fermentation with *Aspergillus* spp. *Biocatalysis and Agricultural Biotechnology* 2020; **25**, 101562.
- [63] J Grzelczyk, P Fiurasek, A Kakkar and G Budryn. Evaluation of the thermal stability of bioactive compounds in coffee beans and their fractions modified in the roasting process. *Food Chemistry* 2022; **387**, 132888.
- [64] EA Setiawan, D Rahardian and Siswanti. The effect of roasting on robusta coffee (*Coffea robusta*) leaves on the chemical and sensory characteristics of refresher beverage. *Jurnal Teknosains Pangan* 2015; **4(2)**, 1-9.
- [65] S Septiana, T Mahatmanto, A Salsabila and WB Sunarharum. The effect of roasting degrees and brewing techniques on the physicochemical and sensory characteristics of anaerobically fermented liberica coffee (*Coffea liberica*). *Trend in Sciences* 2025; **22(6)**, 9819.
- [66] F Bastian, HL Sinaga, A Syarifuddin and A Hambali. Peningkatan cita rasa kopi robusta terdekafeinasi melalui fermentasi ulang menggunakan mucilage tiruan. *Agrointek : Jurnal Teknologi Industri Pertanian* 2024; **18(3)**, 593-604.
- [67] K Sa'diyah, U Ahmad, S Widyantomo and Yusianto. Pengaruh lama perendaman buah dan fermentasi terhadap warna kulit tanduk dan citarasa kopi robusta. *Journal of Industrial and Beverage Crops* 2019; **6(1)**, 33-40.
- [68] A Kinasih, S Winarsih and EA Saati. Karakteristik sensori kopi arabica dan robusta menggunakan teknik brewing berbeda. *Jurnal Teknologi Pangan dan Hasil Pertanian* 2021; **16(2)**, 12-22.
- [69] MR Ramanda, AF Prameswari, MN Ulfa. Effect of Variations of Roasting Temperature on the Physicochemical Properties of Robusta Coffee (*Coffea canephora* L.). *Jurnal Teknik Pertanian Lampung (Journal of Agricultural Engineering)*, **13(2)**, 405-417.
- [70] SN Lessy, L Ega and R Breemer. Pengaruh metode fermentasi dan lama penyangraian terhadap cita rasa kopi tuni asal maluku. *Jurnal Agrosilvopasture-Tech* 2023; **2(2)**, 386-393.
- [71] R Marpaung and Lutvia. Pengaruh lama penyangraian yang berbeda terhadap karakteristik dan mutu organoleptik seduhan bubuk kopi liberika tunggal komposit. *Jurnal Media Pertanian* 2020; **5(1)**, 15-21.
- [72] PJ Barragán, ÓJ Sánchez and JC Henao-Rojas. Evaluation of the growth kinetics of *Lactobacillus plantarum* ATCC 8014 on a medium based on hydrolyzed bovine blood plasma at laboratory and bench-scale levels and its application as a starter culture in a meat product. *Fermentation* 2020; **6(2)**, 45.
- [73] YH Lim, HL Foo, TC Loh, R Mohamad and N Abdullah. Comparative studies of versatile extracellular proteolytic activities of lactic acid bacteria and their potential for extracellular amino acid productions as feed supplements. *Journal of Animal Science and Biotechnology* 2019; **10**, 15.
- [74] DP Larassati, ME Kustyawati, Subeki, D Sartika and S AS. Efek fermentasi basah menggunakan kultur *Saccharomyces cerevisiae* terhadap sifat kimia dan sensori kopi robusta (*Coffea canephora*). *Jurnal Teknik Pertanian Lampung* 2021; **10(4)**, 449-458.
- [75] D Mengesha, N Retta, HW Woldemariam and P Getachew. Changes in biochemical composition of Ethiopian *Coffea arabica* with growing region and traditional roasting. *Frontiers in Nutrition* 2024; **11**, 1390515.
- [76] R Silaban, H Sinaga and T Karo-Karo. Pengaruh jenis kemasan terhadap karakteristik fisikokimia dan sensori kopi arabika gayo dengan metode pengolahan semi basah. *Jurnal Teknologi Pangan dan Hasil Pertanian* 2023; **18(2)**, 13-21.
- [77] MG Wardhana and MS Irwan. Analisis karakteristik kandungan kopi bening (*Clear Coffee*) kabupaten banyuwangi. *Jurnal Agrotek Ummat* 2020; **7(2)**, 65-72.
- [78] R Sharma, P Garg, P Kumar, SK Bhatia and S Kulshrestha. Microbial fermentation and its role in quality improvement of fermented foods. *Fermentation* 2020; **6(4)**, 106.

