

Changes in the Chemical Compound and Sensory Profiles of Liberica Fermentation (*Coffea liberica*) with Cellulolytic Bacteria *Alcaligenes sp* and *Exiguobacterium indicum*

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ABSTRACT

Jambi Province, particularly in Tanjung Jabung Barat Regency, Indonesia, is a crucial producer of Liberica coffee known as Liberika Tungkal Komposit (Libtukom). This variety of Liberica coffee (*Coffea liberica*) thrives in lowland regions, particularly peatlands. It grows best in slightly acidic soil with a pH between 6.0 and 6.5, which helps the plant absorb nutrients and resist pests more effectively. A significant post-harvest method to improve the quality and yield of Liberica coffee involves fermentation using cellulolytic bacteria, *Alcaligenes sp.* And *Exiguobacterium indicum*, found in civet digestive tracts. Fermentation enhances the coffee's flavor, aroma, and quality, mimicking that of civet coffee. The primary objective of this study was to refine the sensory characteristics of Liberica coffee, improve its proximate composition, preserve its bioactive compounds, and boost its antioxidant properties. The fermentation was conducted over 24 hours and 48 hours periods, with roasting temperatures set at light (170 °C), medium (190 °C), and dark (210 °C). The process began with the preparation of bacterial inoculum, followed by fermentation, roasting, and grinding. Sensory evaluation, proximate analysis, and chemical profiling (via FTIR, LC-MS, antioxidant activity, and total phenolic content) were then performed. The findings revealed that fermented Liberica coffee exhibited improved sensory attributes, achieving specialty-grade status at medium roasting temperatures. There was a decrease in moisture and ash content, an increase in fat content, and a reduction in carbohydrate and protein levels. The FTIR analysis showed the preservation of existing compounds, while LC-MS identified bioactive components based on their retention times and molecular weights. Additionally, the fermented coffee demonstrated increased antioxidant activity and total phenolic content compared to non-fermented samples. This study highlights the potential of fermentation technology to enhance the quality and health benefits of Liberica coffee significantly.

Key words: Coffee liberica; chemical compound; fermentation; sensory profile.

1 INTRODUCTION

Jambi Province, particularly in Tanjung Jabung Barat Regency, Indonesia, is a crucial producer of Liberica coffee known as Liberika Tungkal Komposit (Libtukom) with a plantation area of 2,723 hectares and a total production of 1,265 tons per year (Nugroho, 2015). Liberica coffee (*Coffea liberica*) grows in lowland areas and can thrive in peatland. Jambi Province has wetlands dominated by peatlands and swamps reaching 900,000 Ha and is the largest on the island of Sumatra, after Riau and South Sumatra Provinces (Waluyo; Nurlia, 2017). Liberica coffee is a typical peat coffee because it adapts well to peat soil, while Arabica and Robusta coffee cannot grow (Halupi, 2016). This coffee prefers slightly acidic soil with a pH range of 6.0-6.5, which can affect the plant's ability to absorb nutrients and resist pests. Additionally, this coffee has distinctive and unique taste and aroma characteristics (Saidi; Suryani, 2021). Liberica bean has bigger size than Robusta and Arabica, and it also has perceived smell and colour (Duaja; Simatupang; Kartika, 2019).

Liberica coffee is considered to have less commercial value and has less demand in the market compared to Robusta and Arabika (Mubarak et al., 2019). Despite Liberica green

beans possessing a higher antioxidant capacity than Robusta and Arabica green beans, Liberica consumption is still the lowest and the world's Liberica coffee production is only 2% per year, compared to Arabica at 78% per year and Robusta at 20% per year (Saw et al., 2015). Furthermore, there is also a limited study on Liberica coffee on optimizing post-harvesting and fermentation on physicochemical attributes such as chemical compound and overall acceptability of the coffee. The low total production of Liberica coffee is due to less intensive plant maintenance and simple post-harvest processing, resulting in decreased coffee quality (Kitzberger et al., 2020; Wibowo et al., 2021).

Fermentation is biotechnological approach to developing the quality of taste, aroma, and composition of bioactive compounds of coffee beans (Martinez et al., 2019; Wibowo et al., 2021). Coffee bean fermentation aims to understand the transformation of sugar compounds in the parchment layer into alcohol (Lee et al., 2015). Additionally, fermentation can release the coffee aroma at light and medium roasting temperatures. This process helps preserve bioactive compounds (chlorogenic acid, cafestol, kahweol, caffeine, and others) that degrade or break down at high roasting

temperatures (Dark) around 200-230°C (Maligan; Anggono, 2023). Previous studies report that fermentation can improve the aroma and taste of Arabica and Robusta coffee (Martinez et al., 2019). During coffee fermentation, microorganisms produce various metabolites, such as free sugars and free amino acids, which are absorbed into the coffee beans and produce Maillard compounds and volatiles during the roasting process. The increase in sugar and amino acids during fermentation is one of the factors that influences coffee quality (Haile; Kang, 2019). One of the most famous coffees produced through natural fermentation using Luwak digestive microorganisms is Civet Coffee.

One of the fermented coffee products that has developed is civet coffee. Civet coffee is produced through fermentation directly by the civet (*Paradoxurus hermaphroditus*) in its digestive system, assisted by various microbes (cellulolytic, proteolytic, and xylanolytic), this method is known as the in-vivo method (Iswanto et al., 2019; Muzaifa et al., 2023). The fermentation process results in chemical composition changes in coffee beans that can enhance the food flavor quality (Lee et al., 2015; Sipayung et al., 2019; Su et al., 2023). In vitro fermentation was developed to obtain artificial Civet by using microorganisms obtained from Civet digestion, such as yeast and acid bacteria, that enhance the flavour, aroma, and quality of Liberica coffee. Our previous research reported that fermentation using the cellulolytic bacteria *Lactobacillus plantarum* from Civet increased the sensory profile from 7.77 to 8.63 (Latief et al., 2023). Apart from that, fermentation increased the total phenolics and flavonoids of Liberica coffee. In addition, the IC_{50} value of antioxidant activity increased from 72.12 (Strong) to 38.23 (Very Strong). Moreover, present some organic acids in coffee beans are citric acid, quinic acid, and malic acid (Latief et al., 2023).

In this study, we reported in vitro fermentation using another cellulolytic bacteria from Civet, *Alcaligenes sp* (Rahayu; Rahmawati; Kurniatuhadi, 2018) and *Exiguobacterium indicum* (*E. indicum*) (Muzaifa et al., 2023). *Alcaligenes sp* is a Gram-negative bacterium capable of degrading cellulose by producing the enzyme cellulase, which hydrolyzes beta-1,4-glycosidic bonds (cellulose) (Setyati et al., 2023). *E. indicum* is a Gram-positive, rod-shaped, motile cell that produces acid. *E. indicum* hydrolyzes gelatin and cellulose and utilizes glucose, sucrose, and lactose as carbon sources. This bacterium can aid in the fermentation process of coffee beans and help reduce glucose levels in the beans ((Rahayu; Rahmawati; Kurniatuhadi, 2018) The fermentation of Liberica coffee is expected to become a renewed method in post-harvest processing. It can enhance the sensory profile, improve quality, and preserve bioactive compounds. The process helps to break down the bean's natural sugars and acids, leading to a smoother, more complex taste profile that can achieve specialty-grade standards. Furthermore, fermentation

can reduce undesirable elements such as moisture and ash content while boosting more desirable aspects like fat content, which contributes to a richer body and smoother mouthfeel (Latief et al., 2023).

2 MATERIAL AND METHODS

2.1 Material and Instrumentation

The materials used in this study is Green Bean of Liberica coffee (Grade C) obtained from West Tanjung Jabung, Regency, Jambi, Indonesia. Cellulolytic bacteria species *Alcaligenes sp.* and *Exiguobacterium indicum* bacteria were purchased from the National Research and Innovation Agency (BRIN), Indonesia, Nutrient Agar (NA), De Man Rogosa and Sharpe (MRS-Broth) Oxoid, ddH₂O, Buffer Solution, 2,2-diphenyl-2-picrylhydrazyl (DPPH), NaOH, CHCl₃, HCl, concentrated H₂SO₄ solution, KI solution, acetone, Caffeine standard, Ammonia solution, Starch, Na₂S₂O₃, NaSO₄, 60% acetonitrile, CuSO₄, Pb-Acetate, Methanol, NaCl, Bovine Serum Albumin, Acetonitrile, Na₂CO₃, CuSO₄.5H₂O, KNaC₄H₄O₆.4H₂O, and Folin-Ciocalteu reagent (Sigma-Aldrich, St. Louis, MO, USA).

The equipment used includes an analytical balance, hot plate, oven, Probenroster PREIZ roasting machine, Liquid Chromatography Mass Spectrometry (LC-MS), Fourier Transform Infrared (FTIR), UV-Vis spectrophotometer (Thermo-Fisher Orion Scientific AQ8100, Waltham, MA, USA), desiccator, muffle furnace, vortex mixer, incubator, autoclave, bunsen burner, Laminar Air Flow (LAF) hood, and set of Chemical Glass.

2.2 Inoculation and Preparation

5.6 grams of Nutrient Agar was dissolved in 100 mL of distilled water, heated, and homogenized on a hot plate until the solution boiled. The mouth of the Erlenmeyer flask was covered with aluminum foil. It was then sterilized with an autoclave at 120 °C for 155 minutes. After cooling, the NA was poured into petri dishes as needed. Rejuvenation was performed by streaking in a zig-zag pattern on slanted NA medium and incubating at 37 °C for 24 hours (Ananda et al., 2022).

Alcaligenes sp. and *Exiguobacterium indicum* (*E. indicum*) bacteria were taken from colony stock culture and inoculated in 5 mL of MRS Broth, then incubated at 37 °C for 24 hours. Dilution was performed to count the suspension of *Alcaligenes sp.* and *E. indicum*. 1 mL/100 µL of the *Alcaligenes sp.* and *E. indicum* suspension was taken using a micropipette and placed in a sterile test tube containing 9 mL of 0.9% NaCl, then homogenized with a vortex mixer. Dilution was done up to 10⁻⁶. Then, 100 µL was taken with a micropipette and placed in a petri dish containing NA, incubated for 48 hours

at 37°C, in an inverted position. Colonies were counted using a colony counter. The total number of colonies was calculated using the Equation 1.

$$\text{Colony Forming Unit} = \frac{\text{Number of sample colonies}}{\text{Dilution factor} \times \text{Sample Volume}} \quad (1)$$

2.3 Coffee Fermentation

26.1 grams of MRS Broth was dissolved in 500 mL of distilled water, then heated and homogenized on a hot plate, followed by autoclaving at 121 °C for 2 hours. Next, 1 mL of *Alcaligenes* sp. and *E. indicum* starter solution was taken using a micropipette and added to the MRS Broth solution, then incubated for 24 hours at 37 °C. 1 kg of Liberica coffee was placed in a vial or glass container, and then 500 mL of the *Alcaligenes* sp. inoculum solution was added. Fermentation was carried out with two treatments: 24 hours and 48 hours (Table 1). The pH was measured at 0, 24, and 48 hours using a pH meter. After fermentation, the coffee beans were rinsed and dried for 3 days. Once dried, the coffee beans were roasted using a PREIZ Probenroster machine at temperatures of 170°C (Light), 190°C (Medium), and 210 °C (Dark) for 12 minutes (Anhofiah; Rasyid, 2023).

2.4 Sensory Profile

The method used for the coffee sensory test was the Cupping test, according to the standards of the Specialty Coffee Association of America (SCAA), following the SNI 01-2907-2004 standard. The roasted coffee samples were ground to a 20-mesh size and then brewed with 100 mL of hot water at 94-96 °C. The cupping test was carried out by experts

from Indonesian Coffee and Cocoa Research Institute (ICCR) with an assessment of each parameter, namely a score of 6.00-6.75 (good), 7.00-7.75 (very good), 8.00-8.75 (excellent), up to 9.00-10.00 (outstanding) (Isnidayu et al., 2020).

2.5 Proximate Analysis

Moisture: The weight of the plate is recorded, then 2 grams of Liberica coffee powder is weighed using filter paper and placed in an oven for 2 hours at 114 °C. Next, the sample is cooled in a desiccator, and once cooled, it is weighed again along with the plate (Tarigan et al., 2024). The percentage of coffee beans moisture is calculated using the Equation 2.

$$\text{Moisture (\%)} = \frac{\text{Weight after drying} - \text{Weight before drying}}{\text{Weight of the sample}} \times 100\% \quad (2)$$

Total Lipid: The fat content analysis uses the Soxhlet method. The plate is weighed, and 2 grams of Liberica coffee powder is weighed using filter paper, then placed in an oven for 2 hours at 114 °C. Next, the sample is cooled in a desiccator and weighed again along with the plate. The coffee powder in the filter paper is folded and tied with white thread. Then, it is placed in the Soxhlet apparatus with 250 mL of petroleum benzene solvent. The Soxhlet process is carried out at 60 °C for 8 hours. After Soxhlet extraction is complete, the sample is removed, the thread is untied, and it is placed in the oven at 114 °C for 1 hour, then weighed (Berghuis; Maulana, 2023). The fat content is calculated using the Equation 3.

$$\text{Total Lipid} = \frac{\text{Weight of sample (g)} - \text{Weight of fat before extraction (g)}}{\text{Weight of fat after extraction}} \times 100\% \quad (3)$$

Table 1: Sample Codes.

No	Sample Codes	Information
1	CGL	Unfermented Coffee, Roasting Light
2	CGM	Unfermented Coffee, Roasting Medium
3	CGD	Unfermented Coffee, Roasting Dark
4	CA1L	Fermentation Coffee with <i>Alcaligenes</i> sp 24 hours, Roasting Light
5	CA1M	Fermentation Coffee with <i>Alcaligenes</i> sp 24 hours, Roasting Medium
6	CA1D	Fermentation Coffee with <i>Alcaligenes</i> sp 24 hours, Roasting Dark
7	CC1L	Fermentation Coffee with <i>E. indicum</i> 24 hours, Roasting Light
8	CC1M	Fermentation Coffee with <i>E. indicum</i> 24 hours, Roasting Medium
9	CC1D	Fermentation Coffee with <i>E. indicum</i> 24 hours, Roasting Dark
10	CA2L	Fermentation Coffee with <i>Alcaligenes</i> sp 48 hours, Roasting Light
11	CA2M	Fermentation Coffee with <i>Alcaligenes</i> sp 48 hours, Roasting Medium
12	CA2D	Fermentation Coffee with <i>Alcaligenes</i> sp 48 hours, Roasting Dark
13	CC2L	Fermentation Coffee with <i>E. indicum</i> 48 hours, Roasting Light
14	CC2M	Fermentation Coffee with <i>E. indicum</i> 48 hours, Roasting Medium
15	CC2D	Fermentation Coffee with <i>E. indicum</i> 48 hours, Roasting Dark

Ash Content: Ash content is determined using the gravimetric method based on SNI-01-2907-2008. A 2-gram sample is weighed and then ashed in a furnace at 300 °C for 1.5 hours. It is then cooled in a desiccator and weighed. The temperature is then increased to 550 °C for 2.5 hours. Afterward, it is cooled in a desiccator and weighed again. The ash content is calculated according to the equation 4 (Tarigan et al., 2023).

$$\% \text{ Ash content} = \frac{\text{Weight of ash (g)}}{\text{Weight of dry sample}} \times 100\% \quad (4)$$

Carbohydrate Content: Two grams of Liberica coffee powder were added to 10 mL HCl (25%) and refluxed for 3 hours at 100 °C. The solution is then cooled and neutralized with 20% NaOH. It is transferred to a volumetric flask and filtered. A 10 mL portion is placed in an Erlenmeyer flask and boiled, then cooled in an ice bath. Once cooled, KI and H₂SO₄ solutions are added slowly, and the titration is done quickly with 0.1 N Na₂S₂O₃ solution until the color changes to straw yellow (Use starch indicator), and the carbohydrate content is calculated using equation 5 (Tarigan et al., 2023).

$$\text{Total Carbohydrate: } \frac{\text{weight of carbohydrate before extraction} \times \text{Dilution factor}}{\text{Weight of sample (g)}} \times 100\% \quad (5)$$

Total Protein: Protein analysis is determined using the Lowry method. Preparation of Reagents A, B, C, D, and E: Reagent A, Mix 100 grams of Na₂CO₃ in 1000 mL of 0.5 N NaOH (20 grams). Reagent B: Dissolve 1 gram of CuSO₄·5H₂O in 100 mL of distilled water. Reagent C: Dissolve 2 grams of KNaC₄H₄O₅·4H₂O in 100 mL of distilled water. Reagent D, mix 15 mL of Reagent A with 0.75 mL of Reagent B and 0.75 mL of Reagent C. Reagent E, mix 5 mL of Folin-Ciocalteu reagent in 50 mL of distilled water. Preparation of Standard Solution: The standard solution uses Bovine Serum Albumin concentrations of 1, 3, 4, and 5 ppm. Pipette 1 mL of each standard concentration and add 1 mL of Reagent D. Homogenize with a vortex and let it sit for 15 minutes. Then, add 3 mL of Reagent E, homogenize with a vortex again, and let it sit for another 45 minutes. This procedure is repeated for each standard concentration. Sample Preparation: Weigh 0.25 grams of the sample and add 100 mL of distilled water. Stir and filter the solution to obtain the filtrate. Take 1 mL of the filtrate, add Reagent D, and vortex. Let it sit for 15 minutes, add 3 mL of Reagent E, homogenize with a vortex, and incubate at room temperature for another 45 minutes. Measure the sample using a UV-Vis spectrophotometer at a wavelength of 540 nm (Suhendi et al., 2023).

2.6 Chemical Compounds

Coffee Extraction: 1 gram of samples (fermented and unfermented) Liberica Coffee powder extracted with 80%

Methanol (p.a), incubated for 16 hr, then filtered. The extract was then analyzed for total phenols, antioxidant activity, and LC-MS analysis.

Total Phenol: Total Phenol determined by The Folin Ciocalteu Method (with minor modification). A sample solution of 0.025 mL is taken using a micropipette (25 µL), then 0.5 mL of Folin-Ciocalteu reagent and 1 mL of concentrated Na₂CO₃ are added. The mixture is allowed to stand for 3 minutes, then 8.25 mL of distilled water is added. After standing for 30 minutes in a closed room, the absorbance is measured using a UV-Vis spectrophotometer at a wavelength of 725 nm (AHMAD et al., 2015).

Antioxidant Activities: The analysis is conducted using the radical compound 2,2-diphenyl-2-picrylhydrazyl (DPPH) and compared with the standard, gallic acid. A 0.22 mM DPPH solution with a concentration of 1000 ppm is prepared by weighing 0.016 g of DPPH and mixing it with 200 mL of methanol. For the antioxidant analysis, 0.01 mL of the extract is pipetted using a micropipette (10 µL), then 5 mL of the DPPH solution is added using a micropipette (500 µL). The mixture is allowed to stand for 30 minutes in a dark room, then measured with a UV-Vis spectrophotometer at a wavelength of 517 nm. The antioxidant activity of all samples is expressed as IC₅₀, which is defined as the concentration (ppm) of the sample required to inhibit DPPH radicals by 50% (LATIEF et al., 2022).

Bioactive Compounds: The analysis is conducted using a Shimadzu 2020 HPLC equipped with a photo-diode array (PDA) detector and a single quadrupole mass spectrometer (Shimadzu, Japan). The reverse-phase (RP) system is carried out using a C18 column with a mobile phase of acetonitrile (A) and 0.1% formic acid solution (B). A 10 µL coffee extract is used for this analysis. The mobile phase gradient is set as follows: a flow rate of 0.4 mL/min with concentration A from 5 to 30% (0-40 minutes), 30 to 100% (40-45 minutes), isocratic (45-50 minutes), 100 to 50% (50-55 minutes), and 50 to 5% in the last 5 minutes. Component identification with MS is performed using a scan method with a range of 40-1500 m/z. Data interpretation uses HPLC-PDA data at 280 nm for methylxanthine compounds such as caffeine and at 320 nm for phenolic acid compounds. The data is presented qualitatively using the peak area for the identified components (Febrianto; Zhu, 2019).

3 RESULTS

3.1 Fermentation Changes pH and Sensory Profile

The fermentation process in Liberica coffee beans leads to physical changes in the beans. These physical changes can be observed in the beans' physical condition. Table 2 compares pH changes during fermentation. Additionally,

Figure 1 shows a physical shift in coffee beans before and after fermentation.

Table 2: pH Changes during Fermentation.

Fermentation Time	pH Levels	
	<i>Exiguobacterium indicum</i>	<i>Alcaligenes</i> sp
0 hours	6.5	7
24 hours	6	6
48 hours	5	5

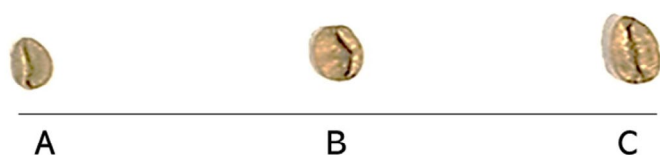


Figure 1: Physical changes; Non-fermentation (A), Fermentation 24 hr (B), Fermentation 48 hr (C).

Coffee beans undergo physical changes, especially those that are not fermented and those fermented freely. The remaining cell wall residues (mucilage) on the parchment skin decrease with longer fermentation times. This allows air to enter the pores of the coffee beans more easily, causing the beans to expand freely and become larger (Suhendi et al., 2023). The breakdown of amino acids during the metabolic process of cellulolytic bacteria results in the accumulation of organic acids and an increase in hydrogen ion (H^+) concentration, leading to a drop in pH levels (Haile; Kang, 2019). By the 24th hour of fermentation, microbes begin to decompose organic compounds and form various carboxylate esters (such as methyl butanoate, cyclohexanol, and sulfur-containing organic compounds) alongside numerous acid compounds, including chlorogenic, quinic, acetic, lactic, malic, phosphate, linoleic, and palmitic acids. These biochemical reactions can lead to the deterioration of coffee beans, producing undesirable odors and further lowering the pH. However, the breakdown of certain acids can sometimes result in pH increases under specific conditions. This intricate process of acid accumulation and degradation influences the sensory and chemical properties of coffee, which is critical for optimizing fermentation conditions to improve quality (Mulyara et al., 2021).

Sensory analysis begins with roasting Liberica coffee at three temperatures: 170°C (Light), 190°C (Medium), and 210°C (Dark) for 12 minutes, followed by grinding the coffee to a medium fineness. The sensory testing method used is the Cupping test according to the standards of the Specialty Coffee Association of America (SCAA). There are 10 parameters, including fragrance (aroma), flavor (a variety of coffee tastes and aromas), aftertaste (taste and aroma impressions left after

swallowing the coffee), acidity/sweetness (categorized as sour or pleasantly crisp/juicy), mouthfeel (light and smooth texture), balance (balance between acidic, sweet, and bitter flavors), uniform cup, clean cup, and overall coffee taste (SNI 01-2907-2008). The results of the sensory test for Liberica coffee are shown in Table 3. The cupping test was carried out by experts with an assessment of each parameter, namely a score of 6.00-6.75 (good), 7.00-7.75 (very good), 8.00-8.75 (excellent), up to 9.00-10.00 (outstanding). Final score by adding up the scores for each parameter (Isnidayu; Sukartiko; Ainuri, 2020).

3.2 Proximate Analysis

The proximate analysis included moisture, fat, carbohydrate, and ash content presented in Table 4. Proximate Analysis is one of the requirements that need to be measured for national coffee standards. The amount of moisture with the maximum limit of SNI-01-3542-2004, which is 7%. Meanwhile, total ash one complies with the SNI-01-2907-2008 standard for ground coffee, which states that ash content should not exceed 5%.

3.3 Total Phenolic and Antioxidant Activities

In the antioxidant analysis of Liberica coffee, the DPPH (2,2-Diphenyl-1-picrylhydrazyl) method is used. Total Phenol and DPPH inhibition tests were conducted on medium-roasted coffee samples. The results of the analysis can be seen in Table 5.

3.4 IR Fingerprint

FT-IR analysis was conducted in this study to determine the effect of different fermentation durations and roasting temperatures on the compounds produced in Liberica coffee fermented for 24 hours and 48 hours. The results of the functional group analysis for Liberica coffee are shown in Figure 2,3,4 and Tables 6a, 6b, 6c.

3.5 Bioactive Compounds

In this study, bioactive compounds were analyzed using LC-MS (Liquid Chromatography Tandem Mass Spectrometry). The principle of the LC-MS instrument involves the separation of sample components based on differences in polarity, followed by the detection of charged ions by the mass spectrometer detector. The analysis results showed 8 compounds with several chromatograms having different retention times, serving as identifiers of specific compounds. Confirmation of compounds is carried out by clarification through detection of molecular weight, maximum peak, spectrum pattern, and retention time of each compound. The results of the bioactive compounds analysis using LC-MS are shown in Figure 5 and Table 7.

Table 3: Sensory Test Results for Fermented and Non-Fermented Liberia Coffee.

Parameters	Samples (Score) ± SD											
	CGL	CGM	CGD	CA1L	CA1M	CA1D	CC1L	CC1M	CC1D	CA2L	CA2M	CA2D
Fragrance	7.5±0	7.5±0	7.5±0	7.5±0.01	7.5±0.01	7.5±0	7.6±0.1	8±1.0	7.25±0.1	7.5±0	7.5±0	7.25±0.1
Flavor	7.5±0	7.25±0.1	7.5±0	7.5±0.02	7.25±0.1	7.5±0	7.6±0.1	8±1.0	7.25±0.1	7.5±0	7.5±0	7.25±0.1
Salt/Acid	7.5±0	7.25±0.1	7.25±0.1	7.5±0.02	7.25±0.1	7.5±0	7.25±0.1	7.5±0.0	7.0±1.7	7.25±0	7.5±0	7.25±0.1
Aftertaste	7.5±0	7.25±0.1	7.5±0.01	7.5±0.01	7.25±0.1	7.25±0.1	7.25±0.1	8.05±1.5	7.25±0.1	7.2±0.1	7.5±0	7.25±0.1
Bitter/Sweet	7.5±0	7.25±0.1	7.75±0.25	7.5±0.01	7.25±0.1	7.75±0.2	7.3±0.2	8.0±1.3	7.5±0	7.25±0	7.5±0	7.25±0.1
Mouthfeel	7.0±0	7.25±0.1	7.25±0.1	7.0±1.7	7.25±0.1	7.25±0.1	7.3±0.2	7.5±0	7.25±0.1	7.25±0	7.5±0	7.25±0.1
Balance	7.0±0	7.25±0.1	7.25±0.1	7.0±1.7	7.25±0.1	7.25±0.1	7.3±0.2	7.5±0	7.75±0.25	7.25±0.1	7.25±0.1	7.25±0.1
Uniform cups	7.50±0	7.25±0.1	7.5±0.01	10±0	10±0.01	10±0	10±0	10±0	10±0	10±0	10±0	10±0
Clean cups	10.0±0	10.0±0.01	10.0±0.01	10±0	10±0.02	10±0	10±0	10±0	10±0	10±0	10±0	10±0
Overall	10.0±0	10.0±0.01	10.0±0.01	7.0±1.7	7.25±0.1	7.25±0.1	7.3±0.2	8.25±1.3	8±1.0	7.75±0.25	7.5±0	7.25±0.1
Final Score	70.0±0 ^a	72.5±0.1 ^a	72.5±0.1 ^a	79±1.1 ^b	80.0±1.1 ^b	79.0±1.1 ^b	80.0±1.0 ^b	83±1.3 ^b	79±1.11 ^b	79±1.11 ^b	80±1.0 ^b	79±1.11 ^b

Superscripts with different lowercase letters in the same column indicate highly significant differences (P<0.05), SD = Standard Deviation.

Table 4: Proximate Analysis Fermented and Non-Fermented Liberia Coffee.

Parameters	Samples											
	CGL	CGM	CGD	CA1L	CA1M	CA1D	CC1L	CC1M	CC1D	CA2L	CA2M	CA2D
Moisture	7.22	6.70	6.34	5.63	6.18	6.89	6.53	6.74	7.00	5.34	5.94	6.55
Fat	9.93	10.40	10.52	12.68	12.53	11.26	2.52	2.69	2.78	12.52	11.38	11.20
Ash	3.84	3.55	2.05	2.31	2.95	3.16	1.00	1.40	2.05	1.45	2.84	2.99
Carbohydrate	77.64	71.24	65.25	41.84	60.47	70.75	48.00	60.25	72.51	41.84	43.33	49.54
Protein	3.36	3.09	1.54	0.52	1.39	1.43	11.44	10.59	10.24	0.52	1.40	1.54

Superscripts with different lowercase letters in the same column indicate highly significant differences (P<0.05), SD = Standard Deviation.

Table 5: Total Phenol and Antioxidant Activity.

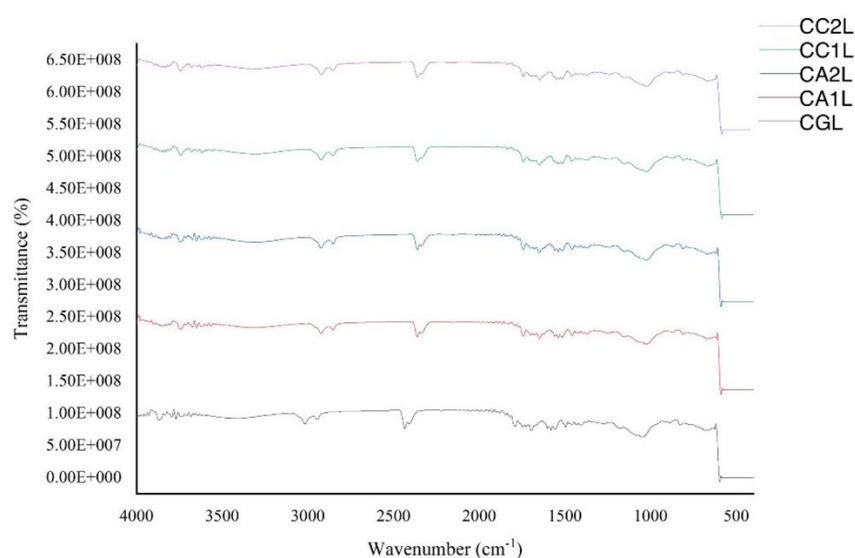
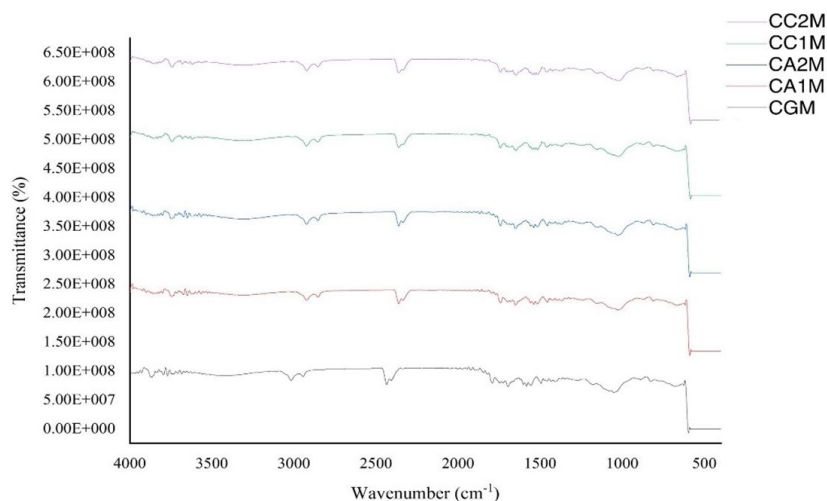
Samples	Total Phenol $\pm$ SD (mg GAE/g)	DPPH Inhibition IC ₅₀ (ppm)	Antioxidant Activity
CGM	11.713 $\pm$ 2.191 ^a	34.05 ^a	Very Strong
CA1M	13.502 $\pm$ 1.372 ^b	31.74 ^b	Very Strong
CA2M	13.643 $\pm$ 0.272 ^b	27.37 ^c	Very Strong
CC1M	13.556 $\pm$ 1.358 ^b	33.39 ^{ab}	Very Strong
CC2M	13.792 $\pm$ 1.509 ^b	27.83 ^c	Very Strong
Ascorbic Acid	-	45.393 ^d $\pm$ 0.000	Very Strong

Superscripts with different lowercase letters in the same column indicate highly significant differences ($P < 0.05$), SD = Standard Deviation.

4 DISCUSSION

4.1 Fermentation Enhanced Sensory Coffee

More intensive chemical changes occur during the fermentation process, which can produce precursor compounds of organic acids, amino acids, and reduced sugars (Balcázar-Zumaeta et al., 2023; Somporn et al., 2011). During the fermentation process, microorganisms produce various types of metabolites. These microorganisms utilize the substrate on the cell wall layer of the coffee beans, making it a precursor that forms features such as body, aroma, and others (Wang et al., 2020). Apart from that, the roasting process can also affect

**Figure 2:** IR Fingerprint Fermented and Unfermented Coffee with Light Roasting.**Figure 3:** IR Fingerprint Fermented and Unfermented Coffee with Medium Roasting.

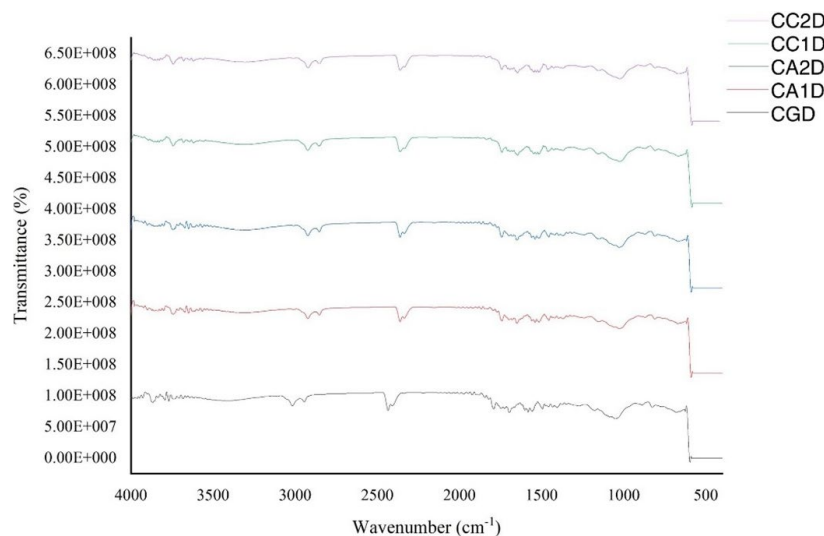


Figure 4: IR Fingerprint Fermented and Unfermented Coffee with Dark Roasting.

Table 6: IR Fingerprint Fermented and Unfermented Coffee with Light Roasting

Structure	Literature (cm ⁻¹)	The Test Result of Absorption (cm ⁻¹)				
		CGL	CA1L	CA2L	CC1L	CC2L
O-H	3650-3200	3639.26	3612.88	3514.11	3649.13	3639.13
N-H	3500-3150	3403.8	3381.59	3396.31	3392.16	3340
C-H	3100-1340	2925.15	2925.21	2866.25	2923.16	2854.69
C-C	1400-1300	1311.65	1319.01	1311.65	1360.85	1355.15
C-O	1300-1000	1150.92	1173	1158.28	1151.33	1132.65
C-N	1280-1360	1305.52	1334.96	1253.98	1347.64	1241.45
C=C	1650-1440	1641.53	1641.53	1644.17	1647.44	1650
C=O	1815-1740	1747.23	1754.60	1739.87	1741.45	1714.88

Structure	Literature (cm ⁻¹)	The Test Result of Absorption (cm ⁻¹)				
		CGM	CA1M	CA2M	CC1M	CC2M
O-H	3650-3200	3409.8	3644.29	3653.98	3649.13	3650
N-H	3500-3150	3412.39	3396.31	3374.23	3374.06	3360.89
C-H	3100-1340	2946.3	2925.21	2917.79	2854.69	2853.34
C-C	1400-1300	1409.79	1319.01	1319.01	1371.75	1371.75
C-O	1300-1000	1160.80	1165.64	1150.92	1126.22	1100.87
C-N	1280-1360	1233.42	1305.52	1342.33	1202.30	1312.65
C=C	1650-1440	1646.8	1641.33	1640.98	1647.44	1639.7
C=O	1815-1740	1754.60	1754.60	1747.23	1764.60	1750

Structure	Literature (cm ⁻¹)	The Test Result of Absorption (cm ⁻¹)				
		CGD	CA1D	CA2D	CC1D	CC2D
O-H	3650-3200	3642.33	3647.85	3558.28	3322.84	3349.23
N-H	3500-3150	3396.31	3352.14	3344.78	3349.84	3434.08
C-H	3100-1340	2925.15	2910.42	2910.42	2880.25	28805.25
C-C	1400-1300	1311.65	1311.65	1311.65	1370.21	1300
C-O	1300-1000	1165.64	1173.00	1150.69	1170.53	1145.51
C-N	1280-1360	1261.34	1268.71	1312.88	1349.48	1329.12
C=C	1650-1440	1641.53	1648.89	1641.10	1649.85	1645.51
C=O	1815-1740	1754.60	1749.87	1747.23	1743.38	1743.87

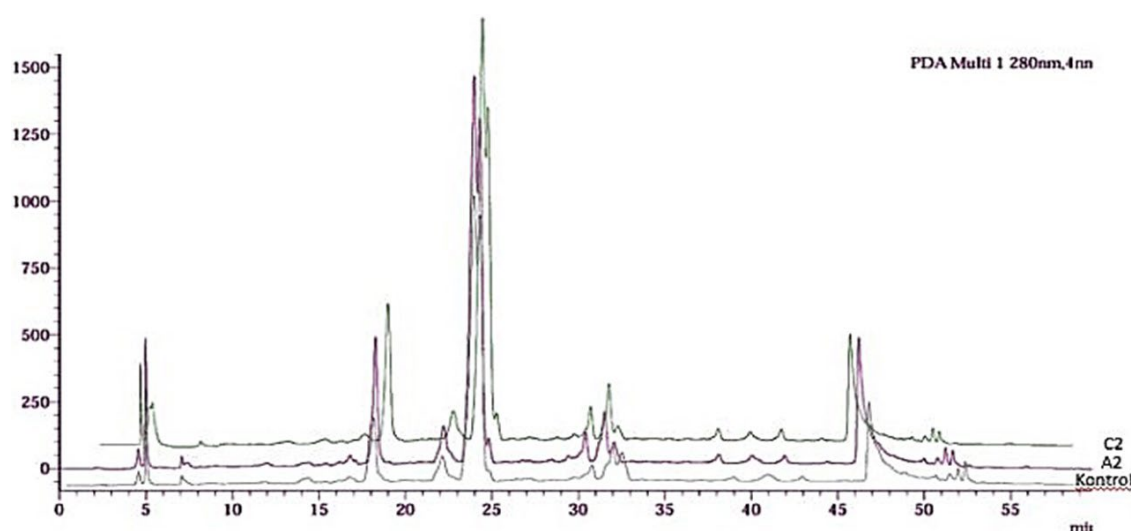


Figure 5: LC-MS Chromatogram Results.

Table 7: Bioactive Compounds Analysis Results with LC-MS.

Component name	Peak maks	MW (gr/mol)	Areas		
			CGM	CA1M	CC1M
n-O-Caffeoylquinic acid (1)	320	354.31	10,056.267	16,540.724	22,603.876
n-O-Caffeoylquinic acid (2)	320	354.31	3,587.369	4,132.718	3,530.617
n-O-Caffeoylquinic acid (3)	320	354.31	42,988.342	51,398.723	61,895.188
Caffeine	280	194.19	26,152.174	24,446.451	24,554.561
n-O-feruloylquinic acid (1)	320	368.3	187,843	467.711	1,551.948
n-O-feruloylquinic acid (2)	320	368.3	4,183.340	5,280.584	6,217.149
n-O-feruloylquinic acid (3)	320	368.3	8,890.272	9,792.084	10,592.256
n-Caffeoyl-1,5-quinolactone	320	336.29	10,581.897	3,889.920	3,046.754

changes in the chemical components of coffee which have an impact on changing its taste (Diviš; Pořízka; Křikal, 2019). In this study show that at medium roasting shows the best condition for Liberica coffee either fermented and non-fermented, the aroma components of the coffee beans begin to emerge. The Maillard reaction occurs at the medium roasting level, producing complex aroma compounds such as fruity, nutty, and caramel, giving the coffee a richer and more interesting flavor (Tamanna; Mahmood, 2015). The spicy taste resulting from bioactive compounds such as caffeine and polyphenols found in raw coffee beans can be reduced with medium processing. Fermentation can produce organic acids such as lactic, acetic, and gluconic acids, which give coffee its acidic taste. Chemical changes become more intense as fermentation continues, producing precursor compounds of organic acids, amino acids, and reduced sugars. During the fermentation process, microorganisms utilize the substrate on the mucilage layer of the coffee beans to produce various types of metabolites. The resulting coffee flavor becomes more delicious as longer fermentation produces a better taste (Mulyara et al., 2021).

4.2 Proximate Changes after Fermentation

Moisture: However, a slight decrease in moisture content is due to fermentation factors, high oven temperatures, and drying. Increased temperature and microbial activity, which enhance enzyme activity and dilute the pulp, cause a decrease in moisture content in coffee as fermentation time increases. Heat causes the pulp to disintegrate and the coffee bean pores to open, leading to faster evaporation of moisture during drying (Schenker et al., 2000). Furthermore, carbohydrates are hydrolyzed by microbes into reducing sugars, ethanol, and organic acids, which require air. Therefore, moisture content should decrease during the fermentation process (Latief et al., 2023). Roasting temperature also plays a role. The longer the roasting process, the faster the heat transfer occurs, accelerating water evaporation. As water molecules move further from the surface, the rate of water evaporation decreases (Anhofiah; Rasyid, 2023).

Fat Content: Cellulolytic bacteria ferment Liberica coffee; as fat breaks down during fermentation, the fat amount decreases. One cause is fat oxidation, which breaks the

backbone bonds (Silaban et al., 2023). During fermentation, air hydrolyzes enzymes in the coffee pulp. As a result, the lipase enzyme breaks down fats into fatty acids, decreasing fat content during fermentation (Wibowo et al., 2021). The roasting process also affects the fat content in the final coffee: the longer and higher the roasting temperature, the more fat is present in the coffee. Lipid content melts during roasting, making coffee beans appear oily and shiny (Budryn et al., 2012). This condition reduces the moisture content of the coffee beans, causing the coffee texture to become hollow and the lipid molecules bound to the water to break down into simpler lipid compounds.

Ash Content: Ash content decreases with longer fermentation times due to the large amount of minerals that dissolve in air and fat during fermentation, which are then lost during drying and pressing, which are then lost during drying and pressing (Prastiwi et al., 2024). Additionally, since minerals are not easily degraded by processing, the decrease in ash content with high roasting temperatures does not have a significant impact (Pamungkas; Masrukan; Sar, 2021), although processing can cause a reduction in minerals by about 3% in food sources, meaning ash content can decrease by more than 0.04%.

Carbohydrates: Carbohydrate content in coffee beans is affected by roasting temperature or roasting. Fermentation temperature increases the amount of complex carbohydrates, such as polysaccharides and sucrose, which then become simple sugars like glucose, fructose, and sucrose (Hu et al., 2020). These simple sugars then participate in Maillard reactions with other compounds, such as amino acids, leading to a decrease in carbohydrate content. Additionally, higher roasting temperatures cause water evaporation and the breakdown of organic compounds, including carbohydrates, in coffee beans. As a result, there is a decrease in total carbohydrate content (Pereira et al., 2017). Different carbohydrate levels, cell wall thickness, and maturity levels greatly influence the distinct characteristics and flavors of coffee beans. Fermentation processes can also lead to a decrease in carbohydrate content (Jokanović et al., 2012). Simple sugars like sucrose, glucose, and fructose are used by bacteria for growth and metabolic activities. During fermentation, bacteria produce enzymes such as amylase, cellulase, and pectinase, which break down complex carbohydrates into simple sugars (Pereira et al., 2017).

Protein: Like carbohydrates and lipids, proteins are complex organic compounds with high molecular weights. Protein content in the fermentation treatment is lower than in the non-fermentation treatment. Table 4 shows how the fermentation process and temperature affect the amount of protein produced. Proteins are broken down into simple amino acids during the fermentation process (Tarigan et al., 2022). The cellulase enzyme in the starter (*Alcaligenes sp* and *Exiguobacterium indicum*), which breaks down protein parts,

causes a decrease in protein content in coffee beans. During washing, proteins soluble in air are carried away. Furthermore, coffee distilled with heat during the roasting process causes its breakdown and denaturation, resulting in a decrease in protein content in the coffee extract. During this process, the protein structure is damaged and breaks into smaller parts. As a result, heating temperature increases and protein content decreases.

4.2 Fermentation Enhanced Either Phenolic Compound and Antioxidant Activity

According to (Balcázar-Zumaeta et al., 2023) coffee fermented at a medium roast level with temperatures around 185°C–205°C has very high antioxidant and phenolic compound content. Chlorogenic acid begins to convert into caffeic acid and quinic acid at the medium roasting level. Additionally, at this level, the Maillard reaction, which produces melanoidin antioxidants, starts to occur perfectly. Fermentation time determines good antioxidant levels because it activates the enzymes responsible for creating antioxidant compounds. During the fermentation process, microorganisms break down complex polyphenols and flavonoids into simpler phenolic compounds, enhancing their capacity as antioxidants. The most abundant polyphenolic compounds in coffee are chlorogenic acid and caffeic acid. Chlorogenic acid accounts for up to 90% of the total phenols found in coffee. Phenolic compounds are reported to show a synergistic relationship with phenolic content; the higher the phenolic content, the higher the antioxidant activity of an extract (Kędzierska-Matysek et al., 2021). The total phenolic and antioxidant presented in Table 6.

Total phenolic content increases along with longer fermentation time. The total phenolic content produced during 48 hours of production indicates that production duration affects the amount of total phenolics generated. Microbial enzymatic activity causes the formation of phenolic compounds (Pereira et al., 2017; Sharma et al., 2020). The microbial enzyme activity, used as a starter in the fermentation process of Liberica coffee, breaks down phenolic compounds bound to cell wall structures such as lignin, cellulose, and proteins through ester bonds into free phenolics, resulting in an increased of phenolic compounds. The total phenolic content produced is also influenced by the roasting process. Moreover, the right temperature and roasting duration increase the overall phenolic content (Somporn et al., 2011). Conversely, roasting that is too short and at too low a temperature does not achieve the ideal solubility, meaning that phenolic compounds have not fully broken down.

4.3 IR Fingerprint of Coffee Fermented and Unfermented

The spectral characteristics of non-fermented coffee, coffee fermented for 24 hours, and coffee fermented for 48

hours are almost the same, based on the data in Tables 7. However, there is a shift in the peaks of the resulting spectra. The absorption in the range of 3743-3302 cm^{-1} indicates -OH functional groups closely related to phenolic compounds in coffee; absorption in the range of 3409-3322 cm^{-1} indicates NH_4^+ functional groups from secondary amines, which are characteristic of caffeine compounds; and absorption in the range of 2925-2853 cm^{-1} indicates aliphatic -CH and -CH₂ functional groups in fatty acids (Simatupang; Herawati; Yuliana, 2023).

The absorption of the C=O group includes amide carbonyl, meaning aldehydes and carboxylic acids, in the range of 1760-1690 cm^{-1} . In the absorption range of 1665-1635 cm^{-1} , the phenyl ring shows C=C functional groups, which is the absorption peak of functional groups for antioxidant compounds such as trigonelline and chlorogenic acid. Amino acid compounds have amine groups with an absorption range of 1522-1507 cm^{-1} with the NCCO group. The absorption range of 1176-1106 cm^{-1} indicates the CO functional groups in alcohols associated with sugars and chlorogenic acid; the absorption range of 1360-1180 cm^{-1} indicates CN functional groups in amines associated with amino acids, as well as CH₃ and CH₂ bonds in aromatic chains and primary and secondary amines, which are characteristic of caffeine compounds in coffee. The main CH function associated with aromatic and alkene compounds is found in the absorption range of 745-735 cm^{-1} (Simatupang; Herawati; Yuliana, 2023). The fermentation process can alter the composition of compounds in coffee, including amino acids, sugars, and others, thus affecting the FTIR spectrum. The most noticeable peak shifts occur in the absorption ranges of 2925-2853 cm^{-1} , which connect the CH groups, and 1176-1106 cm^{-1} , which connect the CO groups (Varão Silva et al., 2021). The roasting process also has an impact, as some coffee compounds are volatile and denature, such as aroma, flavor, and aromatherapy compounds. However, neither fermentation nor roasting temperature significantly affects the composition of coffee compounds; they only alter the resulting spectral peaks.

4.4 Fermentation Changing Chemical and Bioactive Compounds

The compounds identified in this analysis include n-O-Caffeoylquinic acid (1), (2), (3), caffeine, n-O-feruloylquinic acid (1), (2), (3), and n-Caffeoyl-1,5-quinolactone. Compounds such as n-O-Caffeoylquinic acid (1), (2), and (3) are chlorogenic acids known as antioxidants. Caffeine ($\text{C}_8\text{H}_{10}\text{N}_4\text{O}_2$), n-O-feruloylquinic acid (1), (2), and (3), and n-Caffeoyl-1,5-quinolactone are major polyphenolic compounds in coffee besides chlorogenic and caffeic acids. Phenolic acids bound to cell walls can be broken down through microbial fermentation, and using methanol, these active ingredients can be extracted. Additionally, microbial growth during fermentation contributes to this process. According to previous study,

coffee bean fermentation can increase the content of organic acids and phenolic compounds, including phenolic acids, due to enzymatic activity and microorganisms breaking down the cell walls of coffee beans during fermentation (Nizori et al., 2021). *E. indicum* and *Alcaligenes* sp bacteria have the ability to degrade cellulose in coffee beans. Along with previous research showed that because caffeine degrades during fermentation, the caffeine content in coffee decreases with the duration of the fermentation process. These changes lead to the formation of caffeine, which affects the taste and aroma of coffee (Farah; Donangelo, 2006)

5 CONCLUSION

Based on the research results, the sensory scores of coffee fermented with *Alcaligenes* sp. and *E. indicum* increased compared to non-fermented coffee, with scores of 82.0 and 83.0, respectively, falling into the specialty category. The proximate standards of Liberica coffee were maintained, the coffee compounds were preserved in the FTIR analysis, several compounds were detected as bioactive compounds in LC-MS analysis, and the antioxidant and total phenolic content increased in fermented Liberica coffee from three different roasting temperatures

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7 AUTHOR CONTRIBUTION

Conceptual Idea: Indra.L.T; Sutrisno.S.; Madyawati.L; Methodology Design: Indra.L.T; Zairaini.A; Alya.A.P; Data Collection: Zairaini.A; Alya.A.P; Noor.A.F; Data Analysis and Interpretation: Indra.L.T; Zairaini.A; Alya.A.P; Sutrisno.S; ; Noor.A.P; Madyawati.L; Writing and editing: Indra, L.T; Zairaini.A; Alya.A.P; Sutrisno.S

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