



Coffee Improvement by Wet Fermentation Using *Lactobacillus plantarum*: Sensory Studies, Proximate Analysis, Antioxidants, and Chemical Compounds

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Abstract

Fermentation naturally affects the quality and sensory profile of coffee and even improves its nutritional composition. This study aims to evaluate the effect of coffee fermentation using *Lactobacillus plantarum* bacteria on sensory profiles, bioactive compounds, antioxidant activity, and proximate profiles. The study began with the preparation of *Lactobacillus plantarum* (*L. plantarum*) inoculant bacteria, fermentation, total phenol and flavonoid content, caffeine content, chlorogenic acid content, compound analysis, antioxidants, and proximate analysis. Total phenols and flavonoids, caffeine, chlorogenic acid (CA), and antioxidants using the UV-VIS Spectrophotometer instrument. In addition, compound analysis was carried out using GC-MS. This research shows that *L. plantarum* hydrolyzed sucrose into simpler sugars with the invertase enzyme and produce organic acids through glycolysis. Fermentation increased the total phenolics and flavonoids of liberica coffee. In addition, the IC₅₀ value of antioxidant activity increased from 72.12 (Strong) to 38.23 (Very Strong). The most abundant organic acids in coffee beans are citric acid, quinic acid, and malic acid. Fermentation can increase the chemical compounds contained in coffee beans. Liberika coffee taste also increased from 7.77 to 8.63 (Speciality). In addition, fermentation lowers the proximate profile of Liberica coffee. Liberika coffee beans fermented using *L. plantarum* bacteria have better physical quality and taste than original Liberica coffee.

Keywords: Liberica coffee, fermented, *Lactobacillus plantarum*, Sensory, Chemical Compound

1. Introduction

Coffee is one of the plantation commodities with considerable economic value among existing plantation plants and serves an essential function as a source of foreign exchange for the country (Byerlee, 2014). Coffee also acts as a source of income for one and a half million coffee farmers in Indonesia. Coffee is a commodity that has been cultivated in more than 50 countries. Various types of coffee, such as robusta, arabica, gayo, and others, are made to meet the needs of lovers. Liberica coffee was later discovered as a new coffee variety from Tungkai, Jambi (Latief, Heriyanti, et al., 2022; Latief, Muhaimin, et al., 2022). Liberica coffee has the advantages of a larger coffee fruit size and higher productivity than robusta; the price is more

attractive, and it can bear fruit throughout the year with a harvest once a month and the absence of severe pests and diseases (Halupi, 2016). One of the attempts to increase the added value of coffee commodities is to make a coffee product with fermentation technology (Salengke et al., 2019). Coffee bean fermentation is breaking down complex compounds in coffee beans into simpler compounds by involving several microorganisms. This fermentation affects the quality and taste of coffee in coffee beans (Fuferti et al., 2013). One of the famous fermented coffee is the manufacture of civet coffee, from fermentation by civet animals (*Paradoxurus hermaphroditus*) by eating red coffee. Fermentation is carried out in the Civet stomach, shown that enzymes in the civet body ferment the coffee that comes out.

This coffee fruit then undergoes fermentation by various digestive enzymes and is excreted with civet feces as coffee beans still wrapped in horn skin. The penetration of stomach acid and digestive enzymes during fermentation affects the chemical compounds in coffee beans and causes coffee beans to become porous and more brittle. Natural fermentation in the intestine by lactic acid also affects the taste of coffee (Febrianto & Zhu, 2023).

The specialty of its unique taste and origin causes civet coffee to be increasingly in demand among local and world coffee connoisseurs, increasing the demand for these products at phenomenal prices. However, civet cultivation has several disadvantages, including expensive production costs, and can threaten the sustainability of wild mongooses. Therefore, an alternative way of making environmentally friendly civet coffee is needed without reducing the quality of the civet coffee produced. One alternative is to use probiotic microbes that live in the digestive tract of civet animals, considering that the microbes and enzymes contained in the digestive tract are believed to produce fermented coffee to be more unique with a distinctive taste and aroma (Wibowo et al., 2021). Probiotic microbes play a role in the digestion of food in the digestive tract. Probiotic microbes are single cultures or mixtures of live microbes that can help digest animals or humans. Currently, research on the fermentation of Liberica Coffee using *Lactobacillus plantarum* (*L. plantarum*) has not been conducted (Febrianto & Zhu, 2023). Several studies of Liberica coffee fermentation have been conducted using *Saccharomyces cerevisiae*. In addition, there is also research on the wet fermentation of liberica coffee with the addition of α -Amylase. Based on this background, this study aims to evaluate the influence of liberika coffee fermentation using lactic acid bacteria, namely *L. plantarum*, has on its sensory profile, bioactive compound components, antioxidant activity, and proximate profile.

2. Methodology

2.1. Materials

The material used in this study is Liberica Coffee/Original Liberica Coffee (OL) obtained from East Tanjung Jabung Regency, Jambi-Indonesia. *L. plantarum* bacteria was purchase from National Research and Innovation Agency of Indonesia (BRIN), MRSA NA, Aquadest, MRS Borth medium (Merck, Darmstadt, Germany), NaCl 0.9%, methanol, acetone, gallic acid, Folin-Ciocalteu reagent, Na_2CO_3 7%, Aluminum chloride (AlCl_3),

quercetin, sodium acetate, NaOH 1 N, whatmann filter paper, NaSO_4 , CuSO_4 , H_2SO_4 , NaOH 4%, HCl 0.1M, phenolphthalein indicator 1%, KI, $\text{Na}_2\text{S}_2\text{O}_3$ 0.1 N, amylum indicator, HCl 25%, n-hexane, CaCO_3 , CHCl_3 , dichloromethane (DCM), pH paper and 2,2-Diphenyl-1-picrylhydrazylradical (DPPH) (Sigma-Aldrich, Singapura).

2.2. Inoculation of *L. plantarum*

5.6 grams of Nutrien Agar (NA) medium were weighed, put into Erlenmeyer, then dissolved with distilled water until 100 mL were homogenized and heated on a hot plate while stirring until the solution boiled, then covered with aluminum foil. After that, it was sterilized in an autoclave at 121°C for 15 min and then cooled for 30 min. Pour enough liquid into the petri dish and let it stand for 15 min. Several needles of bacterial loops were taken, then incubated by zig-zag scratching on the slanted NA medium, and incubated at 37°C for 24 hr. The stock culture was obtained. 26.1 g of MRS broth was dissolved in 500 ml of dH_2O , homogenized, and heated on a hot plate while stirring until the solution boiled, then autoclaved at 121°C for 2 hr.

2.3. Preparation of Bacterial Suspension

L. plantarum inoculated in 10 mL MRSB medium, and then incubated at 37°C for 24 hr. Calculation of cell population in the suspension of *L. plantarum* done by dilution. Dilution is done by taking as much as 1 mL of suspension *L. plantarum* using a micropipette, putting it into a sterile test tube containing 9 mL of 0.9% NaCl and then vortex. The suspension that has been diluted is taken up to 1 mL and then put into 500 ml of MRS broth starter. Another 100 μL was taken using a micropipette to count colonies, put into a sterile petri dish containing MRSA media, and streaked. The Petri dishes were then incubated for 24 hr at 37°C. Furthermore, the growing colonies are calculated using a colony counter, and the total number of colonies is calculated by the formula 1 (Ananda et al., 2022).

$$N = \sum C / [n_1 + 0.1n_2]d \quad \dots\dots\dots (1)$$

N is the number of bacterial colonies in the plate; $\sum C$ is the sum of plates containing 15 to 300 colonies; n_1 is the number of plates retained in the first dilution; n_2 is the number of plates retained in the second dilution; and d is the first dilution factor. The viable colonies were then converted into log CFU/mL (Ananda et al., 2022).

2.4. Coffee Fermentation and Roasting

Fermentation Liberica Coffee (FL) was conducted by adding 1 kg added into 500 mL of the inoculum solution of *L. plantarum*. Fermentation was carried out at 0, 12, 24, and 36 hr, and each treatment's pH was measured according to room temperature (RT). After soaking, the fermented coffee beans are rinsed, dried in the sun for 6 hr, and dried in an oven at 50–60°C for three days. The dry coffee beans are then roasted (203°C for 2, 4–4 min).

2.5. Sensory Profile

The fermented coffee (FL) sample that has been roasted is then ground to a size of 20 mesh to form a powder brewed with 150 mL of hot water (94–96°C) for the Cupping Test. Assessment of coffee sensory attributes is carried out by experts, including aroma attributes, flavor, aftertaste, acidity, body, balance, uniformity, sweetness, a clean cup, and overall. Panelists rated each sensory detail with a score of 6.00 to 6.75 (good), 7.00 to 7.75 (very good), 8.00 to 8.75 (excellent), and 9.00 to 10.00 (outstanding). The final score is obtained by summing the score of each attribute (Kitzberger et al., 2020).

2.6. Coffee Extraction

One gram of coarse coffee powder was put into a centrifuge tube, 40 mL of methanol/water (50:50), then added HCl to pH two and stirred for 1 hr. They were centrifuged at 2500 rpm for 10 min. The supernatant was separated, and the remaining residue was added to 40 mL of acetone/water (70:30), stirred, and then centrifuged at 2500 rpm for 10 min (extract result 2). The supernatant was separated and mixed with the first and second extraction results, then filtered using Whatman filter paper to obtain the fermented coffee extract. *L. plantarum*. The extract is evaporated in a water bath at a temperature of 90°C until dry (Somporn et al., 2011).

2.7. Total Phenol Content

Preparation of Test Solutions, Pipette 0.8 mL of the fermented Liberica coffee extract solution and add up to 10 mL of methanol to obtain a test solution of 1000 ppm. Then 1 mL was added to 0.5 mL of the reagent Folin-Ciocalteu's reagent and left for 4–8 min. Next, 4 mL of Na solution was added to Na₂CO₃ 7% and shaken until homogeneous. Added up to 10 mL of distilled water, then the solution was incubated at RT for 2 hr, and the absorbance

of the sample was measured with a UV-Vis spectrophotometer (brand mark??, the country of producer). Mer at a maximum wavelength of 744.8 nm. Total phenol was calculated using the linear regression equation of the gallic acid calibration curve that had been measured previously. Preparation of Gallic Acid Standard Solution, A 1000 ppm standard solution was prepared by weighing 10 mg of gallic acid and then dissolving it with methanol P.A. to a final volume of 10 mL. From the highest common resolution, 2.5 mL was pipetted and diluted with methanol P.A. to a volume of 25 mL to produce a concentration of 100 ppm. From this solution, a concentration series of 10 was made; 20; 30; 40, and 50 ppm, which are made by pipetting a resolution of 100 ppm each in the amounts of 1; 2; 3; 4, and 5 mL, put into a 10 mL volumetric flask and diluted with methanol P.A. up to the mark. Each concentration of the gallic acid solution was taken as much as 1 mL and added to 0.4 mL of the reagent Folin-Ciocalteu, then shaken and left for about 4–8 min. Then 4 mL of Na solution was added to Na₂CO₃ 7%, shaken until homogeneous, and added with distilled water up to 10 mL. The mixture was allowed to stand for 2 hr at RT and then the absorbance was measured at a wavelength of 744.8 nm with a UV-Vis spectrophotometer (Farah & Donangelo, 2006).

2.8. Total Flavonoid Content

0.8 mL of the fermented Liberica coffee extract solution add up to 10 mL of methanol to obtain a test solution of 1000 ppm. Then to 1 mL, add 3 mL of methanol, 0.2 mL of AlCl₃ 10%, and 0.2 mL of sodium acetate. Added distilled water up to 10 mL and then allowed to stand for 30 min at RT. The absorbance was measured with a UV-Vis spectrophotometer at a wavelength of 431 nm. Preparation of a Quercetin Standard Solution, A standard solution was prepared to start by dissolving 10 mg of standard quercetin in methanol (p.a) volume of 10 mL and a concentration of 1000 ppm was obtained. From the leading standard solution, 1 mL was pipetted, and then the importance was made up to 10 mL to obtain a concentration of 100 ppm. Then several series of engagements were made, namely 10; 20; 30; 40; and 50 ppm, which were prepared by pipetting a standard solution of 100 ppm quercetin each in the amounts of 1; 2; 3; 4, and 5 mL into a 10 mL volumetric flask. Then to each concentration of 1 mL of quercetin standard solution, 3 mL of methanol P.A., and 0.2 mL of AlCl₃ 10% and 0.2 mL of 1 M sodium acetate and made up to 10 mL of distilled water. After that, the samples

were left for 30 min at RT. The absorbance was measured on a UV-Vis spectrophotometer with a wavelength of 510 nm (Farah & Donangelo, 2006).

2.9. Caffeine Content

1 gram Liberica Original coffee and Fermented Liberica coffee added to hot water, then filtered using a funnel into an Erlenmeyer. The filtrate was put into a separating funnel, and 1,5 grams of calcium carbonate (CaCO_3) was added, which was then extracted with 25 mL of chloroform. The bottom layer was taken, then the extract (chloroform phase) was evaporated with a water bath until the chloroform had evaporated entirely. Caffeine extract from each solvent-free coffee sample was put into a 100 mL volumetric flask and added to the mark. Done dilution by taking 50 mL and adding aquades up to the marked line of 100 ml and homogenizing, then the levels were determined with a UV-Vis spectrophotometer at a wavelength of 200-400 nm. Preparation of the Caffeine Standard Solution, A 1000 ppm mother liquor is prepared by weighing 10 mg of caffeine and putting it into a 10 mL volumetric flask, adding distilled water up to the limit mark. Pipette a solution of 1000 ppm for each amount. Put it in a 10 mL volumetric flask and add distilled water up to the effect (Arwangga et al., 2016).

2.10. Chlorogenic Acid Content

Preparation of Test Solutions, 4.8 mL of coffee extract dissolved in 25 mL of distilled water. The solution was stirred with a magnetic stirrer for 1 hr, accompanied by heating. After that, the answer was filtered to separate the solids. Liquid-liquid extraction used 25 mL of dichloromethane in the sample solution to separate the caffeine from chlorogenic acid. Then stir for 10 min. The aqueous phase and the dichloromethane phase were separated using a separatory funnel. Water containing chlorogenic acid is collected in a beaker, and then the absorbance is measured using a UV-Vis spectrophotometer at a wavelength of 200-400 nm. Preparation of a Chlorogenic Acid Standard Solution, A 1000 ppm mother liquor was prepared by weighing 10 mg of chlorogenic acid and putting it into a 10 mL volumetric flask. Added distilled water up to the mark limit (500 ppm mother liquor). Pipette a solution of 1000 ppm each in the amounts of 0.1; 0.2; 0.3; 0.4, and 0.5 mL (10, 20, 30, 40, and 50 ppm). Put it in a 10 mL volumetric flask and add distilled water up to the mark (Hamdani & Nurman, 2020).

2.11. Volatile Compound

A thick extract of 500 mg of fermented coffee was dissolved into 80 mL of methanol and sonicated for 15 min. The solution is injected into the Gas Chromatography-Mass (GC-MS) instrument as much as one μL . The GC-MS system used is with a flow rate in the column of 1.18 mL/min, with an injection temperature of 80°C, and held for 2 min. The temperature is increased by 10°C/min for 10 min until it reaches a temperature of 270°C. The split ratio used is 100. The scan mode used in the volatile component analysis is classified as full scan mode, with an ion range of 35 to 500 m/z. Data on each peak of molecular fragments obtained from GC-MS were compared with a data source in WILEY7. LIB (Hamdani & Nurman, 2020).

2.12. Determination of Water and Ash Content

Calculate the weight of the porcelain cup first, then put it in the oven at a temperature of 105°C for 1 hr, then remove it from the oven and cool it in a desiccator for 30 min until it reaches RT. 2 g of the sample was put into a porcelain cup. The porcelain cup containing the sample was placed in the oven for 2 hr at 105°C, cooled in a desiccator to RT, then the final weight was weighed. The percentage of water content was calculated by following formula 2.

$$\text{Water (\%)} = \frac{\text{Initial weight} - \text{Final weight}}{\text{Initial weight} - \text{Empty cup}} \times 100\% \dots \dots \dots (2)$$

2.13. Determination of ash content

Put the sample tested for water content into the furnace at a temperature of 525°C until it becomes white ash. After that, it was cooled in a desiccator for 30 min and weighed by following formula 3.

$$\text{Ash (\%)} = \frac{W_2 - W_1}{W_0 - W_1} \times 100\% \dots \dots \dots (3)$$

W2: Final weight (gr); W1: Weight of the empty cup; W0: initial weight

2.12. Determination of protein content

In the destruction stage, 1 g of the sample is weighed, including 7 g of K_2SO_4 and 0.8 g of CuSO_4 . Then put it in a 100mL Kjeldahl flask and add H_2SO_4 concentrated to 15 mL. Then the Kjeldahl flask is heated and destruction can be stopped when a clear greenish solution is obtained. In the distillation stage, the digestion results obtained were then cooled, after which they were diluted with distilled

water up to 100 mL. Add 10 mL of a 30% NaOH solution through the wall of the distillation flask. The distillate flask is installed and connected to the condenser, and the end of the condenser is immersed in the holding liquid. The steam from the boiling liquid will flow through the condenser to the holding Erlenmeyer. The Erlenmeyer container was filled with 10 mL of 0.1 N HCl solution and five drops of PP indicator. In the titration stage, the distillation results collected in an Erlenmeyer are titrated using a 0.1 N NaOH solution. The endpoint of the titration is marked with a colorless solution that turns pink.

2.13. Carbohydrate content

1 g samples added into 50 mL of aquadest and 10 mL of 25% HCl and refluxed for 3 hr at 100°C. The solution is then added PP indicator are added and neutralized with 20% NaOH. Transfer to a 250 mL volumetric flask, then add distilled water to the mark. 10 mL of sample solution, add into 10 mL of luff school, and reflux for 10 min, counting from the start of boiling. It was then cooled in a bath filled with ice. After cooling, 5 mL of 20% KI and 5 mL of NaOH were slowly added to H₂SO₄ 25%. Rapid titration with Na₂S₂O₃ 0.1 N until it changes color to straw yellow, then add three drops of starch indicator. Continue titration until the blue color disappears. Titration was carried out on a blank with the same treatment without using a sample (the sample solution was replaced with distilled water). The volume of Na₂S₂O₃ used is calculated using Formula 4, and the % of Carbohydrates produced is calculated using Formula 5.

$$IN_{\text{that}} = (IN_{\text{blank}} - IN_{\text{sample}}) \times N_{\text{that}} \times 10 \dots\dots(4)$$

$$\text{Carbohydrate (\%)} = \frac{IN_1 \times f.p}{IN} \times 0,90 \times 100\% \dots\dots(5)$$

Note:

IN_{that} : volume Na₂S₂O₃ used (mL)
 IN_{blank} : volume Na₂S₂O₃ used in the blank titration (mL)
 IN_{sample} : volume Na₂S₂O₃ used in sample titration (mL)
 N_{that} : Na₂S₂O₃ used
 IN : sample weight (mg)
 IN_1 : glucose contained per ml of Na₂S₂O₃ used (mg)
 FP : dilution factor

2.14. Determination of fat content

5 g of samples were weighed and put into a paper sleeve lined with cotton. Then plug the paper that already contains the sample with cotton. Then the sleeve is inserted into the soxhlet apparatus. Add n-hexane until n-hexane flowed into the flask, then n-hexane until the piece was submerged. The bath temperature is set at 69°C, which is adjusted

to the boiling point of n-hexane. Extraction was carried out for ± 12 cycles, with a time of ± 6 hr. The extraction results are then distilled to separate the n-hexane from the fat. The formed is then placed in the oven at 105°C for 1 hr, and the fat flask is cooled in a desiccator for 15 min and then weighed.

2.15. Antioxidant activity

DPPH activity of coffee bean extract was evaluated before (control) and after treatment. Briefly, 10 mg samples were dissolved in 25 ml methanol, mixed with 3.8 mL 0.1 mM DPPH solution, and incubated at RT in a dark place for 30 min. After incubation, the absorbance of the sample was measured at 517 nm (Muzdalifa & Jamal, 2019).

3. Results and Discussion

3.1. Coffee Fermentation

In this study, wet processing of coffee was carried out. The damp coffee processing process includes pulping, fermentation, drying, and grinding. Improper processing can result in over-fermented coffee beans. Coffee fermentation procedure by soaking liberica coffee beans into dark glass bottles with lids containing MRS Borth media with a total volume of 500 mL. The bacterial inoculum was transferred as much as 64.5×10^{-7} CFU/mL into a glass vial containing fermentation media. The fermentation process using bacteria affects significant biochemical changes in the biosynthesis of chemical compounds as flavor precursors, including reduced sugar content. This study used *L. plantarum* bacteria that cause changes in liquid pH resulting from fermentation. The initial acidity (pH) in the study was 6. After 12 hr of the fermentation process, the pH did not change, but, during the fermentation process, for 24 hr, there was a decrease in pH to 5. In the 36-hour fermentation process, the pH decreases to 4. The fermentation process influenced by microbial activity, which lowers caffeine levels and pH levels in coffee. Decreased pH levels are affected by the accumulation of organic acids and an increase in the number of H⁺ protons due to bacterial metabolism due to the breakdown of amino acids. After that, the roasting process of original Liberica coffee and Fermented Liberica coffee is carried out with a roasting machine that rotates continuously. In this study, roasting was carried out at a temperature of 203 ° C (*medium roast*), which showed the color of the coffee beans produced was blackish brown and the aroma of thick coffee. In the roasting process, it is possible to change

chemistry which can be seen from the change in the color of coffee beans from bluish-green to blackish-brown (Tarigan et al., 2022).

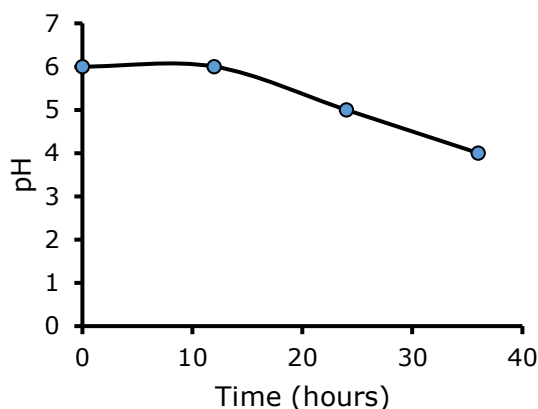


Figure 1. pH changes that occur during coffee fermentation

3.2. Sensory Profile

Three experts conduct sensory testing to determine the main sensory components following the rules of the Specialty Coffee Association (SCA), which has been considered globally as a method of sensory assessment of coffee drinks. The cupping procedure begins by grinding OL beans. Liberika coffee is fermented as a result of roasting and is ground using a Coffee Grinder and brewed using hot water 92-96 °C. Cupping results determined the characteristics of coffee through aroma (dry smell of coffee), flavor (distinctive smell of coffee), body (viscosity), acidity (sour taste), aftertaste (impression of taste), sweetness (sweetness), balance (balance of flavor and aroma), clean cup (clean coffee),

uniformity (consistent taste), overall. The assessment range of each quality attribute is 1-10, where the total value for sensory testing is the result of adding the value of each quality attribute. The results of the assessment conducted by selected experts are presented in Table 1.

Table 1. Original Liberica coffee cupping test score compared to fermented Liberica coffee

Parameter	Samples	
	OL $\pm$ SD	FL $\pm$ SD
Overall	7.67	8.50
Aroma	7.58	8.25
Flavor	7.67	8.00
Aftertaste	7.67	8.00
Acidity	7.77	7.70
Body	7.67	7.90
Balance	7.67	7.90
Uniformity	8.00	10.00
Clean cup	8.00	10.00
Sweetness	8.00	10.00
Final Score	7.77 $\pm$ 0.085	8.63 $\pm$ 0.104

Note: OL = original Liberica coffee, FL = fermented Liberica coffee

Table 1 shows that using *L. plantarum* bacteria in Liberica coffee is the best treatment based on sensory test testing conducted by coffee experts. SCAA determines the limit of specialty coffee if the total taste score of the cupping test results is >80.00 . Fermentation is one of the stages of the primary processing process that significantly determines the quality of coffee brewing (Widyotomo & Yusi, 2013).

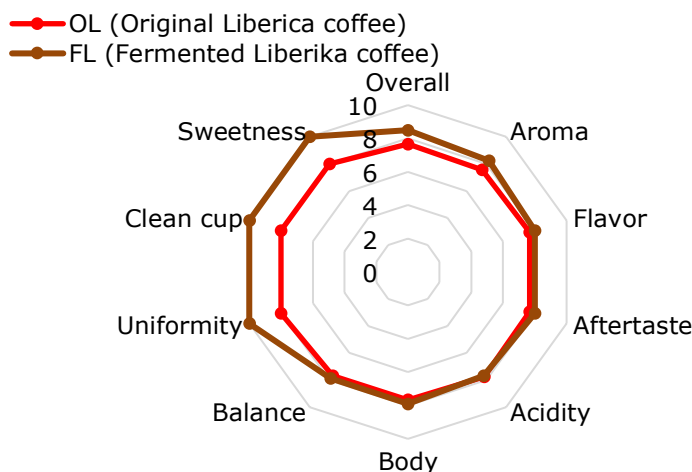


Figure 2. The flavor profile of original Liberica coffee compared to fermented Liberica coffee

Liberica *L. plantarum* coffee is fermented in the best treatment for 36 hr. When fermentation occurs, more intensive chemical changes cause more precursor compounds of organic acids, amino acids, and reducing sugars to form. As a result, more volatile and nonvolatile flavor compounds are created through the Maillard reaction during the roasting process, thus affecting the taste and aroma of brewed coffee. Figure 2 shows the advantages of the taste of *L. plantarum* fermented Liberica coffee in almost all characters. Therefore, it is very reasonable if Liberica *L. plantarum* coffee in this study tastes better than the Original Liberica coffee.

3.3. Total Phenolic and Flavonoids

The test was carried out using two samples, OL samples and FL. The test results of total phenolic levels can be seen in Table 2. Based on the results of Table 2, the results of the total phenolic content show that FL samples have a higher total phenolic value than original liberica coffee. The difference in the total value of phenolics can be caused by bacterial factors used during fermentation. The activity of enzymes produced by bacteria used as starters in the fermentation process, phenolic compounds bound to cell wall structure components such as cellulose, lignin, and proteins through ester bonds will be released into free phenolic, so that it can cause an increase in phenolic compounds. Total phenolics are a series of several compounds in which each combination has an aromatic ring with one or more hydroxyl groups.

The fermentation process involves many types of chemical reactions, such as oxidation which causes the polyphenol yield in this analysis to increase in fermented coffee. High temperatures will cause the total phenol content to be higher because high temperatures can increase the release of phenol compounds on the cell wall. The increase in total phenolic content due to the more elevated the brewing temperature and the longer brewing time effect total phenol active substances extracted by the solvent. Almost all phenol compounds experience damage due to heating temperatures above 85°C. Therefore, I used the highest temperature of 70°C in this study to avoid phenol damage due to high temperatures. Phenolic compounds have various biological effects such as antioxidants, protecting cell structures, anti-inflammatory, and antiseptics (Widyotomo & Yusi, 2013).

Table 2. Total phenolic content in original Liberica coffee and fermented Liberica coffee

Samples	Total Phenolic	Total Flavonoids
OL	42.271 ^a ± 0.04	8.430 ^a ± 0.01
FL	46.385 ^b ± 0.04	12.946 ± 0.03

^{a,b} Superscript with different lowercase letters in the same column shows a very noticeable difference ($p < 0.05$). Table content is discussed if necessary as investigation aim discussed in Introduction section.

The compound used as a standard in determining flavonoid levels is quercetin, a flavonol group flavonoid with a keto group on the C-4 atom and a hydroxyl group on neighboring C-3 and C-5 atoms. The test results of total phenolic levels OL and FL coffee can be seen in Table 2. It is known that the total flavonoids of FL is higher than OL. The increase in flavonoids during fermentation is associated with the enzymatic activity of the microorganism *L. plantarum* in degrading complex polyphenols into small molecules, increasing total phenolics and flavonoids (Arwangga et al., 2016). Microorganisms that play a role in the fermentation process will degrade polyphenolic compounds and complex flavonoids into simpler phenolic compounds so that the number increases overall and increases their potential as antioxidants. The increase in total Bacteri can cause an increase in the number of phenolic compounds resulting from the enzymatic degradation of polyphenolic compounds and complex flavonoids (Bhattacharya et al., 2013). The benefits of flavonoids include protecting cell structure, increasing the effectiveness of vitamin C, being anti-inflammatory, preventing bone loss, and as an antibiotic.

3.4. Total Caffeine and Chlorogenic Acid

They conducted a caffeine level test to find out what percentage of caffeine content was contained in the coffee. Test results of caffeine levels from OL and FL using *L. plantarum* can be seen in Table 3. Based on Table 3, the results of caffeine content show that Original Liberica coffee has a higher caffeine value than *L. plantarum* fermented coffee. The value of caffeine content of OL and FL meets the Indonesian National Standard (SNI 01-3542-2004) is 0.45-2%. The caffeine content in coffee beans is related to many factors, including the plant's growing environment, such as altitude, temperature, rainfall, and nutrients. In addition, the decrease in caffeine

levels is also caused by the activity of lactic acid bacteria, namely *L. plantarum*.

The activity of proteolytic bacteria that produce protease enzymes is high enough to cause caffeine levels in coffee beans to decrease with the longer fermentation process (Chakravorty et al., 2016). During the fermentation process, the mucilage layer that has been lost will make it easier for proteolytic enzymes from *L. plantarum* to enter the cytoplasm and decompose caffeine in coffee beans. Coffee with a low caffeine content is produced from broken-down protein, thereby increasing free amino acids. Cellulolytic and xylanolytic bacteria degrade cellulose and hemicellulose in coffee, so organic acids are formed due to the activity of these bacteria in breaking down sugar. Coffee with low caffeine, in addition to producing good taste and aroma, is also better consumed because consuming low-caffeine coffee will stimulate the nervous system, improve mood and dispel fatigue.

Conversely, high doses will result in insomnia, nervousness, increased heart rate, and blood pressure (Ana Farida, Evi Ristanti, 2013). Caffeine is used as a stimulant to the work of the heart and increases urine production. Caffeine can serve as a stamina generator and pain reliever in low doses. The mechanism of action of caffeine in the body rivals the function of adenosine (one of the compounds that in brain cells can make people fall asleep quickly). Caffeine does not slow down the movement of body cells. Still, caffeine will reverse all the work of adenosine to eliminate drowsiness and bring up a feeling of freshness, a little joy, eyes wide open, a heart beats faster, blood pressure rises, muscles contract, and the heart will release sugar into the bloodstream which will form extra energy (Cappelletti et al., 2014).

Table 3. Total caffeine and chlorogenic acid content in original Liberica coffee and fermented Liberica coffee

Samples	Caffeine	Chlorogenic Acid
OL	0.858 ^a ± 0.01	2.984 ^a ± 0.06
FL	0.821 ^b ± 0.03	2.965 ± 0.04

^{a,b} Superscript with different lowercase letters in the same column shows a very noticeable difference ($p < 0.05$). Table content is discussed if necessary as investigation aim discussed in Introduction section.

Chlorogenic acid (CA) is one of the components contributing to coffee drinks' acidity. Chemically, CA is formed through an esterification reaction between caffeic and quinic acid. The reaction produces several

isomers, the most abundant of which is 5-caffeine quinic acid (5-CQA). CA levels are tested to find out what percentage of CA levels are contained in the coffee. The test was carried out using 2 samples, namely Original Liberica Coffee samples and *L. plantarum* fermentation coffee. Test results of chlorogenic acid levels from original coffee and fermented coffee using *L. plantarum* can be seen in Table 3. It is known that the chlorogenic acid content of fermented coffee is lower than the original liberica coffee. CA levels decrease along with a decrease in caffeine levels. The degradation of chlorogenic acid by bacteria can convert CA into caffeic acid, and CA is broken down into cinnamic acid (Kim et al., 2018). The CA content is based on several factors, such as heating or roasting green coffee beans or "roasted coffee." During the roasting process or roasting coffee, there are physical or chemical changes, as well as the content of the coffee beans (Jokanović et al., 2012).

The roasting process at temperatures above 180-200°C caused significant changes in coffee's chemical composition and biological activity due to the Maillard and Strecker reaction. CA compounds are more susceptible to high temperatures because they are easily hydrolyzed into compounds easily soluble in water and out of coffee beans through hot steam. The roasting process of partial hydrolysis affects chlorogenic acid-producing quinic acid and cinnamic acid, which then undergo decarboxylation into simple phenols with low molecular weight. During roasting, most CA becomes caffeic and quinic acid.

3.5. Volatile Compound

Compound analysis in Liberica coffee is carried out to determine qualitatively and quantitatively the content of coffee compounds before and after fermentation. Based on the results of GC-MS analysis, coffee chromatograms were obtained before fermentation, and 11 peaks were detected, which were produced with varying percentage areas. The peak indicates the type of compound contained by coffee before fermentation. High and low area (%) means the high and low concentration of the identified compound. The higher the peak, the higher the attention of the compound. Based on the chromatogram obtained, it can be seen that the highest peak is owned by Dodecanoic Acid compounds, 1,2,3-Propanetriyl Ester (CAS) Glyceryl Tridodecanoate ($C_{39}H_{74}O_6$), Dodecanoic Acid, 1-(Hydroxymethyl)-1,2-Ethanedyl Ester ($C_{27}H_{52}O_5$), Glyceryl Tridodecanoate ($C_{57}H_{10}O_6$) with a retention

time of 22,450 min and an area percentage of 30.97%.

The results of the GC-MS analysis obtained a chromatogram on FL sample which can be seen in Table 4 and Table 5.

The results of the fermented coffee, chromatogram obtained, 19 peaks which showed the presence of 19 more chemical components contained in coffee after fermentation. There is the highest peak, which is the peak at a retention time of 23,442 min and an area percentage of 28.95%, is a compound Dodecanoic Acid, 1,2,3-

Propanetriyl Ester (CAS) Glycerol Tridodecanoate ($C_{39}H_{74}O_6$), Glycerol Tridodecanoate ($C_{57}H_{10}O_6$), Dodecanamide N - Dodecyl -N- (Trifluoroacetyl) - (CAS) N-Trifluoroacetyl- N Dodecyl dodecanamide ($C_{26}H_{48}F_3NO_2$) which is an organic acid compound formed due to bacterial activity that occurs when fermented. The most abundant organic acids in coffee beans are citric acid, quinic acid, and malic acid. Fermentation can increase the chemical compounds contained in coffee beans.

Table 4. Volatile Compound in OL coffee

No.	R. Time (Minute)	Area (%)	Formulas	Compound Name
1	22.308	7.98	$C_{39}H_{74}O_6$	Dodecanoic Acid, 1,2,3-Propanetriyl Ester (CAS) Glycerol Tridodecanoate
			$C_{27}H_{52}O_5$	Dodecanoic Acid, 1-(Hydroxymethyl)-1,2-Ethanediy Ester
			$C_{57}H_{10}O_6$	Glycerol Tridodecanoate
2	22.450	30.97	$C_{39}H_{74}O_6$	Dodecanoic Acid, 1,2,3-Propanetriyl Ester (CAS) Glycerol Tridodecanoate
			$C_{27}H_{52}O_5$	Dodecanoic Acid, 1-(Hydroxymethyl)-1,2-Ethanediy Ester
			$C_{57}H_{10}O_6$	Glycerol Tridodecanoate
3	22.625	10.30	$C_{49}H_{94}O_6$	Eicosanoic Acid, 2-[(1-Oxohexadecyl) Oxy]-1-(CAS) Glycerol-2-Eicosanoate-1,3 Dihexadecanoate
			$C_{57}H_{10}O_6$	Glycerol Tridodecanoate
			$C_{27}H_{52}O_5$	Dodecanoic Acid, 1-(Hydroxymethyl)-1,2-Ethanediy Ester
4	22.800	7.16	$C_{39}H_{74}O_6$	Dodecanoic Acid, 1,2,3-Propanetriyl Ester (CAS) Glycerol Tridodecanoate
			$C_{57}H_{10}O_6$	Glycerol Tridodecanoate
			$C_{35}H_{66}O_5$	2-Lauro-1,3 Didecoin
5	22.875	5.79	$C_{39}H_{74}O_6$	Dodecanoic Acid, 1,2,3-Propanetriyl Ester (CAS) Glycerol Tridodecanoate
			$C_{57}H_{10}O_6$	Glycerol Tridodecanoate
			$C_{27}H_{52}O_5$	Dodecanoic Acid, 1-(Hydroxymethyl)-1,2-Ethanediy Ester
6	22.942	9.84	$C_{39}H_{74}O_6$	Dodecanoic Acid, 1,2,3-Propanetriyl Ester (CAS) Glycerol Tridodecanoate
			$C_{57}H_{10}O_6$	Glycerol Tridodecanoate
			$C_{35}H_{66}O_5$	2-Lauro-1,3 Didecoin
7	23.092	15.82	$C_{39}H_{74}O_6$	Dodecanoic Acid, 1,2,3-Propanetriyl Ester (CAS) Glycerol Tridodecanoate
			$C_{57}H_{10}O_6$	Glycerol Tridodecanoate
			$C_{26}H_{48}F_3NO_2$	Dodecanamide, N-Dodecyl-N-(Trifluoroacetyl)-(CAS) N-Trifluoroacetyl-N-Dodecyl dodecanamide
8	23.150	8.41	$C_{39}H_{74}O_6$	Dodecanoic Acid, 1,2,3-Propanetriyl Ester (CAS) Glycerol Tridodecanoate
			$C_{57}H_{10}O_6$	Glycerol Tridodecanoate
			$C_{26}H_{48}F_3NO_2$	Dodecanamide, N-Dodecyl-N-(Trifluoroacetyl)-(CAS) N-Trifluoroacetyl-N-Dodecyl dodecanamide
9	23.283	2.35	$C_{39}H_{74}O_6$	Dodecanoic Acid, 1,2,3-Propanetriyl Ester (CAS) Glycerol Tridodecanoate
			$C_{49}H_{94}O_6$	Hexadecanoic Acid, 2-[(1-Oxododecyl) Oxy]-1,3-Propanediyl Ester (CAS) Glycerol-2-Laurate-1,3-Dipalmitate
			$C_{55}H_{106}O_6$	Eicosanoic Acid, 2-[(1-Oxohexadecyl) Oxy]-1-(CAS) Glycerol-2-Eicosanoate-1,3 Dihexadecanoate
10	23.442	1.03	$C_{39}H_{74}O_6$	Dodecanoic Acid, 1,2,3-Propanetriyl Ester (CAS) Glycerol Tridodecanoate
			$C_{14}H_{26}O_2$	Dodecanis Acid, Ethenyl Ester (CAS) Vinyl Dodecanoate
			$C_{17}H_{36}$	Tetradecane, 2,6,10-trimethyl-(CAS) 2,6,10-Trimethyltetradecane
			$C_{35}H_{66}O_5$	2-Lauro-1,3 Didecoin

11	23.542	0.35	C ₃₉ H ₇₄ O ₆	Dodecanoic Acid, 1,2,3-Propanetriyl Ester (CAS) Glyceryl Tridodecanoate
			C ₁₄ H ₂₆ O ₂	Dodecanoic Acid, Ethenyl Ester (CAS) Vinyl Dodecanoate
			C ₂₇ H ₅₂ O ₅	Dodecanoic Acid, 1-(Hydroxymethyl)-1,2-Ethanedyl Ester

Table 5. Volatile Compound in FL coffee

No.	R. Time (Minute)	Area (%)	Formulas	Compound Name
1.	19.817	0.65	C ₃₉ H ₇₄ O ₆	Dodecanoic Acid, 1,2,3-Propanetriyl Ester (CAS) Glyceryl Tridodecanoate
			C ₅₇ H ₁₀ O ₆	Glyceryl Tridodecanoate
			C ₂₇ H ₅₂ O ₅	Dodecanoic Acid, 1-(Hydroxymethyl)-1,2-Ethanedyl Ester
2.	19.933	0.91	C ₃₉ H ₇₄ O ₆	Dodecanoic Acid, 1,2,3-Propanetriyl Ester (CAS) Glyceryl Tridodecanoate
			C ₅₇ H ₁₀ O ₆	Glyceryl Tridodecanoate
			C ₂₇ H ₅₂ O ₅	Dodecanoic Acid, 1-(Hydroxymethyl)-1,2-Ethanedyl Ester
3.	20.158	1.80	C ₃₉ H ₇₄ O ₆	Dodecanoic Acid, 1,2,3-Propanetriyl Ester (CAS) Glyceryl Tridodecanoate
			C ₅₇ H ₁₀ O ₆	Glyceryl Tridodecanoate
			C ₂₇ H ₅₂ O ₅	Dodecanoic Acid, 1-(Hydroxymethyl)-1,2-Ethanedyl Ester
4.	20.483	4.08	C ₃₉ H ₇₄ O ₆	Dodecanoic Acid, 1,2,3-Propanetriyl Ester (CAS) Glyceryl Tridodecanoate
			C ₅₇ H ₁₀ O ₆	Glyceryl Tridodecanoate
			C ₂₇ H ₅₂ O ₅	Dodecanoic Acid, 1-(Hydroxymethyl)-1,2-Ethanedyl Ester
5.	20.625	0.60	C ₃₉ H ₇₄ O ₆	Dodecanoic Acid, 1,2,3-Propanetriyl Ester (Cas) Glyceryl Tridodecanoate
			C ₅₇ H ₁₀ O ₆	Glyceryl Tridodecanoate
			C ₂₇ H ₅₂ O ₅	Dodecanoic Acid, 1-(Hydroxymethyl)-1,2-Ethanedyl Ester
6.	20.750	2.25	C ₃₉ H ₇₄ O ₆	Dodecanoic Acid, 1,2,3-Propanetriyl Ester (CAS) Glyceryl Tridodecanoate
			C ₂₇ H ₅₂ O ₅	Dodecanoic Acid, 1-(Hydroxymethyl)-1,2-Ethanedyl Ester
7.	20.817	1.04	C ₃₉ H ₇₄ O ₆	Dodecanoic Acid, 1,2,3-Propanetriyl Ester (CAS) Glyceryl Tridodecanoate
			C ₄₉ H ₉₄ O ₅	Eicosanoic Acid,2-[(1-Oxohexadecyl) Oxy]-1-(CAS) Glyceryl-2-Eicosanoate-1,3 Dihexadecanoate
			C ₁₄ H ₂₆ O ₂	Dodecanoic Acid, Ethenyl Ester (CAS) Vinyl Dodecanoate
8.	20.942	0.80	C ₃₉ H ₇₄ O ₆	Dodecanoic Acid, 1,2,3-Propanetriyl Ester (CAS) Glyceryl Tridodecanoate
			C ₅₇ H ₁₀ O ₆	GLYCERYL TRIDODECANOATE
			C ₄₉ H ₉₄ O ₅	Eicosanoic Acid,2-[(1-Oxohexadecyl) Oxy]-1-(CAS) Glyceryl-2-Eicosanoate-1,3 Dihexadecanoate
9.	21.158	1.36	C ₃₉ H ₇₄ O ₆	Dodecanoic Acid, 1,2,3-Propanetriyl Ester (CAS) Glyceryl Tridodecanoate
			C ₁₉ H ₄₀	Nonadecane (CAS) N-Nonadecane
			C ₁₄ H ₂₆ O ₂	Dodecanoic Acid, Ethenyl Ester (CAS) Vinyl Dodecanoate
10.	21.283	2.06	C ₃₉ H ₇₄ O ₆	Dodecanoic Acid, 1,2,3-Propanetriyl Ester (CAS) Glyceryl Tridodecanoate
			C ₅₇ H ₁₀ O ₆	GLYCERYL TRIDODECANOATE
			C ₁₄ H ₂₆ O ₂	Dodecanoic Acid, Ethenyl Ester (CAS) Vinyl Dodecanoate
11.	21.383	1.15	C ₃₉ H ₇₄ O ₆	Dodecanoic Acid, 1,2,3-Propanetriyl Ester (CAS) Glyceryl Tridodecanoate
			C ₁₄ H ₂₆ O ₂	Dodecanoic Acid, Ethenyl Ester (CAS) Vinyl Dodecanoate
			C ₅₇ H ₁₀ O ₆	GLYCERYL TRIDODECANOATE

12.	21.475	1.55	C ₃₉ H ₇₄ O ₆	Dodecanoic Acid, 1,2,3-Propanetriyl Ester (CAS) Glyceryl Tridodecanoate
			C ₄₇ H ₉₀ O ₆	Hexadecanoic Acid, 2-[(1-Oxododecyl)Oxy]-1,3-Propanediyl Ester Glyceryl-2-Laurate-1,3-Dipalmitate
			C ₅₇ H ₁₁₀ O ₆	Glyceryl Tridodecanoate
13.	22.633	0.75	C ₃₉ H ₇₄ O ₆	Dodecanoic Acid, 1,2,3-Propanetriyl Ester (CAS) Glyceryl Tridodecanoate
			C ₁₄ H ₂₆ O ₂	Dodecanoic Acid, Ethenyl Ester Vinyl Dodecanoate
			C ₂₆ H ₄₈ F ₃ NO ₂	Dodecanamide, N-Dodecyl-N-(Trifluoroacetyl)- (Cas) N-Trifluoroacetyl-N-Dodecylidodecanamide
14.	22.908	21.38	C ₃₉ H ₇₄ O ₆	Dodecanoic Acid, 1,2,3-Propanetriyl Ester (Cas) Glyceryl Tridodecanoate
			C ₂₇ H ₅₂ O ₅	Dodecanoic Acid, 1-(Hydroxymethyl)-1,2-Ethanedyl Ester
			C ₅₇ H ₁₁₀ O ₆	Glyceryl Tridodecanoate
15.	23.008	3.70	C ₃₉ H ₇₄ O ₆	Dodecanoic Acid, 1,2,3-Propanetriyl Ester (Cas) Glyceryl Tridodecanoate
			C ₅₇ H ₁₁₀ O ₆	Glyceryl Tridodecanoate
			C ₃₅ H ₆₆	2-Lauro-1,3-Didecoin
16.	23.092	8.54	C ₃₉ H ₇₄ O ₆	Dodecanoic Acid, 1,2,3-Propanetriyl Ester (Cas) Glyceryl Tridodecanoate
			C ₃₅ H ₆₆	2-Lauro-1,3-Didecoin
			C ₅₇ H ₁₁₀ O ₆	Glyceryl Tridodecanoate
17.	23.308	16.41	C ₃₉ H ₇₄ O ₆	Dodecanoic Acid, 1,2,3-Propanetriyl Ester (CAS) Glyceryl Tridodecanoate
			C ₃₅ H ₆₆	2-Lauro-1,3-Didecoin
			C ₅₇ H ₁₁₀ O ₆	Glyceryl Tridodecanoate
18.	23.442	28.95	C ₃₉ H ₇₄ O ₆	Dodecanoic Acid, 1,2,3-Propanetriyl Ester (CAS) Glyceryl Tridodecanoate
			C ₅₇ H ₁₁₀ O ₆	Glyceryl Tridodecanoate
			C ₂₆ H ₄₈ F ₃ NO ₂	Dodecanamide, N-Dodecyl-N-(Trifluoroacetyl)- (CAS) N-Trifluoroacetyl-N-Dodecylidodecanamide
19.	23.617	2.02	C ₃₉ H ₇₄ O ₆	Dodecanoic Acid, 1,2,3-Propanetriyl Ester (CAS) Glyceryl Tridodecanoate
			C ₄₇ H ₉₀ O ₆	Hexadecanoic Acid, 2-[(1-Oxododecyl)Oxy]-1,3-Propanediyl Ester (CAS) Glyceryl-2-Laurate-1,3-Dipalmitate
			C ₅₅ H ₁₀₆ O ₆	Eicosanoic Acid, 2-[(1-Oxohexadecyl) Oxy]-1-(CAS) Glyceryl-2-Eicosanoate-1,3 Dihexadecanoate

3.6. Proximate Analysis

Water content can affect the durability of materials during storage against microorganism attacks. Ground coffee is expected to have a low water content because it can increase the resistance of ground coffee from damage caused by microorganisms during storage. Each sample is used as much as 2 g during testing. The moisture content test results can be seen in Table 6. The moisture content in all samples meets the requirements of SNI ground coffee 01-3542-2004 with a maximum limit of 7%. The result shown that the water content of fermented coffee is smaller and better than the water content of original liberica coffee. This is because fermentation using *L. plantarum* bacteria is very influential on coffee. When fermentation occurs, bacteria cause a temperature rise that can change the mucus from the coffee beans to be diluted, the pores of the coffee beans open, and the evaporation process occurs. This fermentation process will

also convert mucus into alcohol from hygroscopic sugar compounds in the slime. This results in coffee beans that are dried and processed into ground coffee will experience a decrease in water content with an increasing number of bacterial inoculum. The fermentation process of coffee by lactic acid bacteria will result in coffee absorbing a lot of water, thereby increasing the weight of fermented coffee beans. Microbial activity in this fermentation will remodel macromolecules such as starch, fat, and protein into simpler compounds so that water is able to diffuse into cells contained in coffee beans. However, coffee will shrink again during the drying process. Coffee will also shrink during the roasting process (Vollmer et al., 2017).

Ash content describes the mineral content or all organic matter in the product remaining after ashing. Ash is an inorganic residue from a combustion process at a temperature of 6500 C. A material contains primarily organic

matter and water; the rest is inorganic compounds called minerals and ash. Each sample is used as much as 2 g during testing. The ash content test results can be seen in Table 6. The ash content in all samples meets the requirements of SNI ground coffee 01-3542-2004 with a maximum limit of 5%. Based on Table 6, it is known that the ash content value in fermented coffee samples is smaller than the original coffee. The addition of *L. plantarum* bacteria and fermentation time affect the ash content of ground coffee. The longer the fermentation in the coffee beans, the more during the fermentation process, there is an overhaul of the components of substances in the coffee beans due to the fermentation process producing heat. In the fermentation process, organic substances are decomposed into water and oxygen, but organic matter does not leave a residue in the form of ash. High ash content indicates a high mineral content. Coffee has several mineral contents: potassium, potassium, calcium, magnesium, and non-metallic minerals, namely phosphorus and sulfur.

Protein levels in this study were determined using the Kjeldhal method, also called crude protein content, because it is bound to N compounds instead of proteins. Protein has a role in determining the quality of coffee because the lower the protein content, the less bitter the coffee taste. The Kjeldhal method's working principle is that the sample's proteins and organic components are destroyed using sulfuric acid and a catalyst. The result of destruction is neutralized using alkaline solutions and through distillation. Distillation is accommodated in a solution of boric acid. Furthermore, the ions formed are titrated using an HCl solution. Protein analysis consists of three stages: digestion, distillation, and titration. The results of testing the protein content of original coffee and fermented coffee using *L. plantarum* are in Table 8. Based on Table 6, it is known that the highest protein content is Original coffee. This is because the longer the fermentation time, the protein content will decrease. It decreases protein levels due to the breakdown of proteins into amino acids. The protein content contained in fermented coffee is the total amount of protein material used and the protein of *L. plantarum* bacteria present in it. *L. plantarum* bacteria that are part of the added lactic acid bacteria will utilize nitrogen and carbon sources to live and multiply. The more the number of bacterial cultures, the higher the protein content because most of the constituent components of bacteria are proteins (Vollmer et al., 2017).

Fat content testing results of original coffee and fermented coffee using *L. plantarum* (Table 6). It is known that the highest fat content is authentic coffee. The longer the fermentation time and the greater the concentration of *L. plantarum* bacteria, the relative fat content decreases. Overweight decomposition factors generally occur during roasting, namely fatty acids in coffee beans that are still contained will evaporate, while the fat moves to the coffee beans' surface (Bahroni et al., 2019). The long fermentation of civet coffee can reduce fat content because it is related to the enzymes owned in the pulp of the coffee fruit skin, with the hydrolysis of water, so that the lipase enzyme in coffee mucus seeds and coffee fruit flesh, decomposes fat into fatty acids (Hanifah & Kurniawati, 2013).

In contrast, fatty acids have reduced reactions with strong alkalis from the beginning, i.e., NaOH is one of them. Fermentation is basically the breakdown of proteins, fats, and carbohydrates by bacteria, so in fermented coffee, there are simple fractions such as amino acids, fatty acids, and glucose. Lipase enzyme hydrolysis of triglycerides in fats will produce fatty acids and fats.

Carbohydrate components contained in coffee beans are essential for forming aroma components, primarily through low molecular weight caramelization and Maillard reactions with amino acids. The carbohydrate content of fermented coffee is smaller than the original coffee. This is due to the use of carbohydrates as an energy source for the growth of *L. plantarum* bacteria (Table 6). The decrease in carbohydrate levels is caused by the breakdown of polysaccharides into simple sugars. Glucose is one of the simple sugars that produce organic acids. In addition, microbes during fermentation require energy from overhauling organic or non-organic compounds, provided mainly by carbohydrate compounds (Sharma et al., 2020). The most widely used sources of energy are carbohydrates (CH_2O) and hydrocarbons (CH_2). Carbohydrates in the form of crude fiber are organic materials that contribute carbon elements to the bioconversion process of food substances in the fermentation process carried out by bacteria to produce energy. The fermentation process can break down carbohydrates by bacteria so that there are simple compounds such as glucose in fermented coffee.

Table 6. Proximate Analysis of Original and Fermented Liberica Coffee

Samples	Water content (%)	Ash content	Protein	Lipid	Carbohydrate
OL	4.30	4.17	17.61	10.17	5.94
FL	3.14	3.44	12.41	9.11	3.02

3.7. Antioxidant Activity

Coffee rich in antioxidant compounds from the hydroxycinnamic acid group and other biological compounds that have significant antioxidant potentials, such as caffeine, nicotinic acid, and trigonelline. Antioxidant activity is described with an IC_{50} which means the ability to dampen 50% of free radicals at a given concentration. The smaller the IC_{50} , the greater its ability to capture free radicals, meaning that the material has high antioxidant activity (Yashin et al., 2013). Based on the study's results, there is a difference in IC_{50} value between Original Liberica coffee and Fermented Liberica coffee (Table 7).

Table 7. Antioxidant activity of original Liberica coffee and fermented Liberica coffee

Samples	IC_{50}	Antioxidant
OL	72.12	Strong
FL	38.23	Very Strong

^{a,b} Superscript with different lowercase letters in the same column shows a very noticeable difference ($p < 0.05$). Table content is discussed if necessary as investigation aim discussed in Introduction section.

The IC_{50} Fermented Liberica coffee is smaller than the Original Liberica coffee. Therefore, Fermented Liberica coffee has a functional level of antioxidant power compared to Original Liberica coffee. This can occur due to bacterial activity that occurs during fermentation. *L. plantarum* bacteria change the chemical composition in coffee to be better than Original Liberica coffee.

4. Conclusion

Fermentation using *L. plantarum* increased the total phenolic and flavonoids of Liberica coffee. In addition, the IC_{50} value of antioxidant activity increased from 72.12 (Strong) to 38.23 (Very strong). The most abundant organic acids in coffee beans are citric acid, quinic acid, and malic acid. Fermentation can increase the chemical compounds contained in coffee beans. Liberica

coffee flavor also increased from 7.77 to 8.63 (Speciality). In addition, fermentation lowers the proximate profile of Liberica coffee. Liberica coffee beans fermented using *Lactobacillus plantarum* bacteria have better physical quality and taste than Original Liberica coffee.

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