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Enhancement of Liberica Coffee Quality by Wet Fermentation using *Bacillus subtilis*

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ABSTRACT. Coffee is a tree species in the Rubiaceae family and Coffea genus. One way to enhance the value of coffee commodities is by using fermentation technology. Fermentation can use probiotic bacteria found in mongoose digestive tracts, creating coffee with a unique taste and aroma. *Bacillus subtilis* is one of these bacteria, offering an alternative to those from mongoose. This study aimed to analyze the effects of fermentation using *B. subtilis* bacteria on the physicochemical and sensory properties of Liberica coffee. The study method included bacteria preparation, coffee fermentation, and testing. The samples' total phenolics, flavonoids, caffeine, and chlorogenic acid contents were estimated. The analysis was performed using UV-Vis and GC-MS spectrophotometers. The results revealed that the fermentation process using *B. subtilis* improved the sensory and physical qualities of the fermented Liberica coffee. The total values of phenolic and flavonoid contents and antioxidants of the fermented Liberica coffee were higher than that of the original coffee. GC-MS analysis exhibited compounds in the fermented coffee in which the highest was N-Dodecyl-N-(trifluoroacetyl)dodecanamide ($C_{26}H_{48}F_3NO_2$), with a retention time of 23.73 minutes and an area percentage of 18.96%. Moreover, the caffeine and chlorogenic acid of the fermented Liberica coffee was lower than that of the original coffee.

INTRODUCTION

Coffee is a tree-shaped plant species that belongs to the family Rubiaceae and the genus Coffea (Byerlee, 2014). Indonesia is currently reported as the fourth largest coffee-producing country in the world after Brazil, Vietnam, and Colombia, and the total coffee exported reaches 67% (Prajanti *et al*., 2020). Robusta and Arabica coffee are more prevalent in Indonesia and come from the highlands, around 1,300 to 2,000 meters above sea level (Bilen *et al*., 2023). One type of coffee with a distinctive taste is Liberica and Excelsa, which come from the lowlands of western and central Africa (Latief *et al*., 2022). Nevertheless, Liberica coffee (*Coffea sp.*) is a type of biodiversity that has thrived in Indonesia recently.

Liberica coffee is an agro-industrial commodity that has the potential to be developed in East Tanjung Jabung Regency, Jambi Province. It is an interesting coffee plant because it can adapt to various peatlands with high acidity levels. Because of this adaptability, Liberica coffee is also known as peat coffee. Meanwhile, other coffees, such as Arabica and Robusta, cannot grow in peat soils (Campuzano-Duque *et al*., 2021). The potential and uniqueness of Liberica coffee have not been able to attract the public's attention. Therefore, Liberica coffee needs to be processed further in post-harvest and pre-harvest.

In general, one strategy to improve the value and quality of coffee commodities is to make special products through fermentation biotechnology (Sharma *et al*., 2020). Coffee fermentation can affect the physical quality and taste of a product. Besides, it can change the product's physical and chemical composition (Wibowo *et al*., 2021). Furthermore, fermentation can increase the total polyphenol and flavonoid components, which correlate with antioxidant activity (Agustriana *et al*., 2023). Natural fermentation (in vivo) can occur by utilizing living creatures. One well-known example of a product applying in vivo fermentation is Luwak (mongoose) coffee (Putri *et al*.,

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2015). Fermentation in the mongoose's stomach is a perfect fermentation process since the microbes in the mongoose's stomach can work perfectly and play a role in coffee degradation (Munandar *et al*., 2022).

However, the coffee fermentation process using mongoose animals has several weaknesses, especially its impact that endangers wild mongoose populations in nature (Towaha and Rubiyo, 2016). Besides, it is also considered to torture the mongoose and threaten the animal's survival (Usman *et al*., 2015). Alternatively, fermentation can be conducted in vitro using microorganisms like those obtained from mongoose digestion. It is done to produce coffee with a unique taste like Luwak coffee but with a more environmentally friendly, effective, and efficient process (Towaha and Rubiyo, 2016).

In vitro fermentation can use microbes obtained from mongoose digestion. The microbes generally found in mammalian digestion are the Lactic Acid Bacteria, Bacillus, and other probiotic groups. One probiotic bacterium that can be employed as a fermenting agent for civet digestion is *B. subtilis*. The effects of the fermentation conducted using a group of probiotic bacteria need to be studied concerning the coffee taste quality and its chemical compounds (Ngamnok *et al*., 2023).

Probiotic bacteria can be employed in fermentation because they can positively affect other organisms and fermented products (Kechagia *et al*., 2013). *B. subtilis* is a type of probiotic bacteria that has the potential to be developed in the biotechnology industry. Previous research revealed that fermentation using *B. subtilis* could improve the digestibility of soybean proteins and the health benefits of aglycone isoflavones. Besides, fermentation using these bacteria could form various health molecules of vitamins, reduce carbohydrates-induced intestinal flatulence, and positively regulate intestinal commensals that support the absorption of these bacteria in the intestine (Gao *et al*., 2022). Moreover, various *B. subtilis* can produce extracellular enzymes that degrade pectin, produce catalase enzymes, and hydrolyze carbohydrates (Nasanit and Satayawut, 2015). *B. subtilis* bacteria are considered promising as coffee cherries fermenting microorganisms. As the fermenting microorganisms, they work primarily at the pectinase stage or peeling and the reduction of the slime layer on coffee cherries, which have been proven to accelerate and improve fermentation quality (Oumer and Abate, 2017). Additionally, using *B. subtilis* as a starter culture in Arabica coffee fermentation could improve the sensory quality of coffee, namely aroma, taste, aftertaste, body, balance, and overall (Febrianto and Zhu, 2023).

Recently, only a few studies are available on the use of starters in coffee fermentation, which comes from the genus *Bacillus* which has various enzymatic activities such as pectinolytic, cellulolytic, amylolytic, and proteolytic (Chi *et al*., 2016). Therefore, this research aimed to study the effects of Liberica coffee fermentation using *B. subtilis* on its sensory and physicochemical properties.

RESEARCH METHODS

Chemicals and Instrumentation

The materials were Liberica coffee obtained from East Tanjung Jabung Regency, Jambi, and *Lactobacillus plantarum* bacteria from the National Research and Innovation Agency (BRIN). The chemicals were MRS-NA and MRS Broth, purchased from Merck, Germany. The others were distilled water, NaCl 0.9%, methanol, acetone, gallic acid, Folin Ciocalteu reagent, Na_2CO_3 7% (w/v), aluminum chloride ($AlCl_3$), Quercetin, Sodium Acetate, NaOH 1N, Whatman filter paper, Na_2SO_4, $CuSO_4$, H_2SO_4, NaOH 4% (w/v), HCl 0.1M, phenolphthalein (PP) indicator 1%, KI, $Na_2S_2O_3$ 0.1 N, amylum indicator, HCl 25%, n-hexane, $CaCO_3$, chloroform, and dichloromethane. They were purchased from Sigma-Aldrich, USA.

The equipment included a set of rotary glassware (BUCHI), a set of bacterial culture tools, an autoclave, an incubator, a colony counter, a coffee roaster, a centrifuge, an oven, a desiccator, a Kjeldahl flask, and a coffee grinder. The instruments used for analysis were a Gas Chromatography-Mass Spectrometry (GC-MS) (Shimadzu QP2010 SE) and a UV-Vis Spectrophotometer (Thermo-Fisher Orion Scientific AQ8100, Waltham, MA, USA). The data obtained were analyzed using one-way analysis of variance (ANOVA) with a confidence level of 95% to see significant differences between treatments. Duncan's post hoc follow-up test was employed to determine total phenolic, flavonoids, caffeine, and chlorogenic acid content.

Inoculation and Preparation of *Bacillus subtilis*

Agar (NA) media as much as 5.6 g was dissolved in 1000 mL of distilled water. It was then homogenized and heated at 80 – 90 °C. Next, the media was sterilized using an autoclave at 121 °C for 15 min. Furthermore, the *B. subtilis* bacterial inoculum was incubated in autoclaved media at 37 °C for 24 hours to obtain culture stock. The

B. subtilis culture stock was inoculated into 10 mL of sterilized MRS Borth medium and then incubated at 37 ℃ for 24 hours. The number of cell populations was estimated in CFU by diluting 1 mL of cell in 9 mL of 0.9% NaCl to produce a cell suspension. 1 mL of cell suspension was diluted into 500 mL MRS Borth starter. Then, the number of colonies was counted with a micropipette. The colonies were placed in a sterile petri dish containing MRSA and then incubated at 37 ℃ for 24 hours. The growing colonies were computed with a colony counter, and their total number was calculated by Equation (1) (Ananda *et al*., 2022).

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

N represents the number of colonies, ∑C is the number of plates containing 15 to 300 colonies, n 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 dilution factor.

Coffee Fermentation

One thousand grams of green Liberica coffee beans were placed into a glass flask and then soaked in 500 mL of *B. subtilis* inoculum. The fermentation was conducted at room temperature for 12, 24, and 36 hours. During the fermentation process, changes in pH were observed using an Eutech pH meter (Thermo Scientific, Waltham, MA, USA). After fermentation, the green coffee beans were washed thrice using sterile water. The fermented coffee beans were dried in an oven with an adjusted temperature of 45 ℃ to maintain the moisture content at 10% (Adrianto *et al*., 2020).

Sensory Properties

Samples of fermented and unfermented Liberica coffee (later called Fermented Liberica Coffee or FLC and Original Liberica Coffee or OLC) were ground with a size of 20 mesh. The powder was then brewed with 150 mL of hot water at about 94 – 96 °C for a cupping test. The assessment of coffee sensory properties was conducted by experts from the Jambi Coffee Cupper teams. The evaluation referred to the Specialty Coffee Association of America (SCAA), scoring between 1 and 10 for each test variable. A score of 1 was the lowest, and 10 was the highest (Erskine *et al*., 2022).

Coffee Extraction

One gram of Liberica coffee powder was added with 40 mL of methanol/water (50:50 v/v), and then HCl was added until pH 2 was obtained. Subsequently, it was vortexed for 3 minutes and centrifuged at 2500 rpm for 10 minutes. The supernatant was separated and put into a vial (Filtrate 1). The remaining residue (pellet) was mixed with 40 mL of acetone/water (70:30) and vortexed for 3 minutes. The result was centrifuged at 2500 rpm for 10 minutes (Filtrate 2). The final supernatant was separated and mixed with Filtrates 1 and 2 until a liquid extract was obtained (Somporn *et al*., 2011).

Total Phenolic Content

The total phenolic content in fermented and original Liberica coffee was measured with the modified Folin-Ciocalteu method. Briefly, 80 µL of sample extract was mixed with 80 µL Milli-Q water-diluted Folin reagent and 200 µL Milli-Q water. This mixture was incubated in a dark condition for 5 minutes at room temperature. Afterwards, 4 µL of 10% (w/w) sodium carbonate solution was given, and the plate was further incubated for 1 h under the same dark condition. Meanwhile, gallic acid in ethanol solution ranging from 0 – 50 µg/mL was prepared and employed to generate a calibration curve. To eliminate the sample's colour interference on absorbance, a mixture of sample and water was used as blank. After incubation, the absorbance was measured at 745 nm using a UV/Vis spectrophotometer (Thermo-Fisher). The results were expressed as mg of gallic acid equivalents (GAE) per gram of coffee (mg GAE/g) ± the standard deviation (SD) (Haile and Kang, 2019).

Total Flavonoid Content

The total flavonoid content in fermented and unfermented coffee beans was measured by following previous studies with slight modifications. Briefly, 80 µL of sample extract was mixed with 80 µL of 2% aluminium chloride and 120 µL of sodium acetate solution (50 g/L) and incubated for 30 min at room temperature. Afterwards, the absorbance was measured at 431 nm using a calibration curve of quercetin in an alcohol solution with

concentrations ranging from 0 – 50 µg/mL. The results were obtained by subtracting it from the blank and expressed as milligrams of quercetin equivalents (QE) per gram of coffee (mg QE/g) ± the standard deviation (SD) (Haile and Kang, 2019).

Caffeine Content

Caffeine content was determined by following previous studies with several modifications. For the standard solution, the commercial chemical standard of caffeine was used and dissolved in 70% EtOH previously filtered using a 0.2 μm filter. The solutions were prepared into several concentrations, ranging from 0 to 25 µg/mL. They were uniformly dissolved by stirring using a magnetic stirrer for 30 min. The UV/Vis absorbance spectra of the solutions were recorded at 200 – 400 nm to determine their molar extinction coefficient. The same procedure was then conducted on the standard caffeine with ethanol solvent. The results of standard solution measurements were applied to plot the regression curve.

One gram of Liberica coffee sample was stirred into 150 mL of hot distilled water. The mixture was then filtered using a 0.2 μm filter, and the filtrate was added with 1.5 g of Calcium Carbonate ($CaCO_3$). The result was then extracted by 25 mL $CHCl_3$ to produce coffee extract. The coffee caffeine extract was poured with 100 mL of distilled water. 50 mL of the solution was taken and then diluted by adding distilled water until the total volume reached 100 mL. The extract was then homogenized, and the caffeine content was determined using UV-Vis spectrophotometry at 200 – 400 nm wavelengths. The caffeine concentration and CGA were computed by applying the Lambert-Beer law (Navarra et al., 2017).

Chlorogenic Acid Content (CGA)

For the standard solution, commercial chemical standard CGA was applied and dissolved into 70% EtOH previously filtered using a 0.2 μm filter. The standard solutions were prepared into several concentrations, ranging from 0 to 50 µg/mL (Awwad *et al*., 2021). The UV/Vis absorbance spectra of the standard solutions nm were recorded at 200 – 400 to determine their molar extinction coefficients. For the sample solutions, 4.8 mL of coffee extract was dissolved in 25 mL of distilled water. The solution was stirred and heated for 1 hr. The sample solution was filtered and extracted using 25 mL of dichloromethane (stirred for 10 min). The extract was then separated by a separator funnel. The absorbance was measured using a UV-Vis spectrophotometer at 200 – 400 nm wavelengths to determine their molar extinction coefficients (Navarra *et al*., 2017).

Volatile Compound Analysis

The volatile compounds were analyzed using the GC-MS instrument. First, 500 mg of fermented coffee beans (*Coffea liberica*) was dissolved in 80 mL of methanol and sonicated for 15 minutes. 1 µL of the solution was injected into the GC-MS instrument. The GC-MS system used a flow rate of 1.18 mL/min and an injection temperature of 80 °C. The temperature was increased by 10 °C/min for 10 minutes until it reached 270 °C. The split ratio was 100. The scan mode was classified as full scan mode, with an ion range of 35 – 500 m/z. The results of the GC-MS analysis were spectra. Each peak in the spectra represented molecular fragments that were then compared with a data source in WILEY7. LIB (Zhang et al., 2022).

Proximate Analysis

Determination of moisture content

A petri dish was weighed and placed in the oven at 105 °C (1 day), then was cooled after 1 hour. Two grams of samples were placed in the petri dish and heated in an oven at 105 °C for 2 days. The final mass was weighed, and the percentage of water content was calculated using Equation (2).

$$\%\text{Moisture content} = \frac{\text{Initial weight} - \text{Final weight}}{\text{Initial weight} - \text{Empty cup}} \times 100\% \quad (2)$$

Determination of ash content

Two grams of the samples were placed into a petri dish. It was then heated at 550 °C in a furnace for 3 hours until the sample was gray. The samples were then removed from the furnace and cooled in a desiccator for 30 minutes (Indonesia National Standard, SNI 2014). The final mass was weighed. The ash content was calculated by Equation (3).

$$\%\text{Ash content} = \frac{\text{Final weight} - \text{Weight of the empty cup}}{\text{Initial weight} - \text{Weight of the empty Cup}} \times 100\% \quad (3)$$

Determination of Protein Content

One gram of sample, 7 g of K_2SO_4, and 0.8 g of $CuSO_4$ were placed into a 100 mL Kjeldahl flask. Concentrated H_2SO_4 as much as 15 mL was added to the mixture. Then, the Kjeldahl flask was heated, starting with low heat (room temperature). After a while, the temperature was increased until it boiled. The destruction process could be stopped when a clear greenish-coloured solution was obtained. In the distillation process, the digestion product was diluted with 100 mL of distilled water. NaOH 30% solution of as much as 10 mL was then given through the inner wall of the distillation flask. The distillate flask was installed and connected to the condenser, and then the condenser tip was immersed in the holding liquid. Steam from the boiling liquid would flow through the condenser to the Erlenmeyer reservoir. 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 accommodated in Erlenmeyer were titrated using 0.1 N NaOH solution. The endpoint of the titration was marked with a colorless solution becoming pink. The blanks were titrated using the same treatment without samples (SNI, 2014). The protein content was estimated using Equation (4).

$$\%\text{Protein content} = \frac{(V1-V2)}{\text{IN}} \times 0.014 \times \text{CF} \times \text{DF} \times 100\% \quad (4)$$

IN is the sample weight (gram), V1 is the 0.1 N NaOH used in the sample titration (mL), V2 is the 0.1 N NaOH used in the blank titration (mL), N is NaOH normality, FK is the conversion factor, and DF is the dilution factor.

Determination of Carbohydrate Content

One gram of the samples was added with 50 mL of distilled water and 10 mL of 25% HCl. The solution was refluxed for 3 hours at 100°C and then cooled. Tree drops of PP indicator were given. Next, it was neutralized with 20% NaOH (pH checked with universal indicator). After that, the solution was transferred to a 250 mL measuring flask, and distilled water was poured to the limit mark. Next, 10 mL of the sample solution was added with 10 mL of Luff Schoorl. The solution was then refluxed for 10 minutes (the time was calculated from the start of boiling). Afterward, it was cooled in a bath filled with ice. After cooling, 5 mL of 20% Kl and 5 ml of 25% H_2SO_4 were slowly poured. The solution was immediately titrated with 0.1 N $Na_2S_2O_3$ solution. When the straw turned yellow, three drops of amylum indicator were given. Then, the titration process was continued until the blue colour disappeared. Titration was also done on blanks with the same treatment without using samples (sample solutions were replaced with distilled water). The carbohydrate content was estimated using Equations (5) and (6).

$$IN_t = (IN_B - IN_s) \text{ x } N_t \times 10 \quad (5)$$

$$\%\text{ Carbohydrate} = \frac{IN_1 \text{ x DF}}{\text{IN}} \times 0.9 \times 100\% \quad (6)$$

IN_t is the volume of $Na_2S_2O_3$ used (mL), IN_B is the volume of $Na_2S_2O_3$ used in the blank titration (mL), IN_s is the volume of $Na_2S_2O_3$ used in sample titration (mL), and IN_1 is the glucose contained per ml of $Na_2S_20_3$ used (mg).

Determination of Fat Content

Five grams of the samples were placed in a paper container lined and covered with cotton. Then, the sleeve was inserted into the soxhlet tool. The n-hexane flowed into the flask until the sample was submerged. Then, the soxhlet tool was connected to the condenser. The bath temperature was set at 69 °C, which was adjusted to the boiling point of n-hexane. The extraction was completed for ± 12 cycles with a time of ± 6 hr. The extraction results were then distilled to separate n-hexane from the fat. The fat formed was then baked at 105 °C for 1 hour. Next, the fat flask was cooled in a desiccator for 15 minutes and weighed. The remaining mass was expressed as fat weight computed as fat content using Equation (7).

$$\%\text{Fat} = \frac{\text{Sample weight} - \text{Weight of fat before extraction}}{\text{Weight of fat flask after extraction}} \times 100\% \quad (7)$$

Antioxidant activity

The antioxidant activity of coffee beans before and after fermentation was evaluated using the DPPH method employing the organic chemical compound 2,2-diphenyl-1-picrylhydrazyl. Briefly, 10 mg of the samples was dissolved in 25 mL of methanol. It was then mixed with 3.8 mL of 0.1 mM DPPH solution and incubated for 30 minutes at room temperature. After incubation, the absorbances of the samples were measured at 517 nm wavelength (Latief *et al*., 2022).

RESULT AND DISCUSSION

Coffee Fermentation

Fermentation employs certain enzymes from microorganisms to eliminate or add chemical compound components. Microbes can produce various enzymes and organic acid metabolite compounds, which can reduce the pH value. This research employed *B. subtilis* for coffee fermentation. The pH values after several fermentation periods are shown in Figure 1. It exhibits that the pH values decreased gradually as the longer fermentation. The reduced pH value is due to bacterial activity, which produces organic acids resulting from the decomposition by enzymes during Liberica coffee fermentation. *B. subtilis* produces protease enzymes that degrade proteins into amino acids and lipase enzymes into fatty acids (Balachandran *et al*., 2021). The longer fermentation produced more organic acids, decreasing the pH value. In prior studies, the microflora of coffee fermentation can significantly generate metabolites such as ethanol. The ethanol is then oxidized into organic acid components such as acetic acid, oxalic acid, lactic acid, etc., so that coffee's aroma is enhanced. Oxalic acid is the highest level, followed by acetic and lactic acid, causing the pH to decrease or increase acidity (Correa *et al*., 2014).

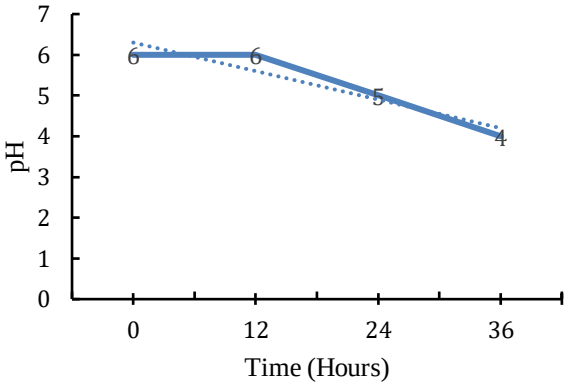

Figure 1. Decrease in pH values as the longer fermentation.

Sensory Properties

Roasted FLC and OLC were coarsely ground using a Latina Grinder and then brewed with hot water at 92 – 96 °C. The cupping test was done based on the Specialty Coffee Association of America (SCAA). The analysis involved fragrance/aroma (dry aroma of coffee grounds/aroma after brewing), flavour (taste and aroma), body (thickness), acidity, aftertaste (taste impression in the mouth), sweetness (sweet taste), balance (balance of taste and aroma), clean cup (cleanness of taste characteristics), uniformity (taste consistency), and overall. The taste of coffee was significant, so the experts acted as a measuring tool that must be sensitive and consistent. The score results from the cupping test can be seen in Figure 2.

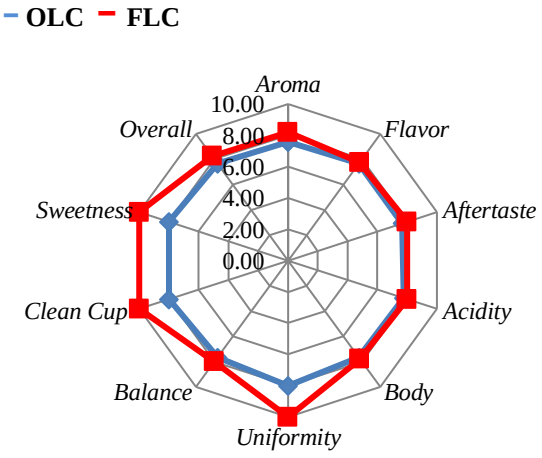

Figure 2. The results of the sensory properties.

Compared to the OLC sample, in almost all attributes, the results showed that the final score of the FLC sample was 8.60 (Figure 2). Based on the SCAA, coffee can be categorized as a specialty when the final taste score obtained from the cupping test is ≥80.00. Meanwhile, the OLC sample was included in the premium coffee because the final score was <80. Probiotic coffee has a more potent aroma than Luwak coffee. Therefore, it is only natural that the fermented Liberica coffee in this study tasted slightly better than the original Liberica coffee. A chemical reaction occurs during coffee fermentation. This reaction helps form coffee flavour characters by forming precursor compounds, including reducing sugars, amino acids, and organic acids (Devi *et al*., 2021). Chlorogenic acid is an important compound that affects taste, smell, and flavour formation when roasting coffee.

Total Phenolics Content

The total phenolic content was determined using the Folin-Ciocalteu method. The principle of this method is the oxidation of phenolic hydroxyl groups. The Folin-Ciocalteu reagent will reduce heterology acids to molybdenum-tungsten complexes and oxidize phenolics. During the reaction, the phenolic hydroxyl group reacts with the Folin-Ciocalteu reagent to form a phosphomolybdic acid complex, which can be detected with a UV-Vis spectrophotometer. As a comparison, gallic acid was used because it is a phenolic compound derived from hydroxybenzoic acid, a simple and stable phenolic acid with antioxidant activity (Cindrić *et al*., 2011). The total phenolic contents in FLC and OLC are displayed in Table 1.

Table 1. Total phenolics content in fermented and original Liberica coffee

Samples	Total Phenolics Content (mgGAE/G)		Average ± SD (mgGAE/g)
	I	II	
OLC	42.297	42.245	42.271 ± 0.036
FLC	45.214	45.266	46.240 ± 0.036

Note: OLC (Original Liberica Coffee) and FLC (Fermented Liberica Coffee)

It shows that OLC had a lower total phenolic content than FLC, namely 42.271 ± 0.036 mgGAE/g and 46.240 ± 0.036 mgGAE/g, respectively. The lower phenolic content represents increased antioxidant activity during fermentation. During the process, phenolic compounds and organic acids are easily hydrolyzed. Consequently, the solubility of phenolic compounds and organic acids in fermented coffee is enhanced, improving the antioxidant activity. Changes in total phenol levels might be due to the formation of phenolic compounds as a result of bacterial enzymatic reactions that degrade other matrix components due to enzymatic activity in bacteria. The activity of enzymes produced by bacteria used as starters in the fermentation process can increase phenolic compounds bound to structural components of cell walls, such as cellulose, lignin, and protein, through ester bonds and are released into free phenolics. Besides, the level of stability of polyphenolic compounds during storage can be influenced by several external factors, such as air, storage temperature, and light (Cao *et al*., 2021).

Total Flavonoids Content

The total flavonoid contents in FLC and OLC samples were measured using a UV-Vis spectrophotometer, where the standard used was quercetin. $AlCl_3$ was added to form ortho hydroxy and hydroxy ketones complexes to determine the total flavonoid content. The total flavonoid contents in OLC and FLC samples shown in Table 3.

Table 3. Total flavonoid contents in fermented and original Liberica coffee

Samples	Total Flavonoids Content (mgQE/g)		Average ± SEM (mgQE/g)
	I	II	
OLC	8.376	8.484	8.430 ± 0.076
FLC	12.462	12.570	12.516 ± 0.076

Note: OLC (Original Liberica Coffee) and FLC (Fermented Liberica Coffee)

Data in Table 3 reveals that the total flavonoid content in the FLC sample was higher than that of the OLC sample, namely 12.516 ± 0.076 mgQE/g and 8.430 ± 0.076 mgQE/g, respectively. This is caused during fermentation, when bacteria break down complex polyphenolic and flavonoid compounds into simpler phenolic compounds and increase their overall number (Morales, 2020). Roasting at high temperatures and for a long time will reduce flavonoids due to degradation, namely enzymatic and non-enzymatic oxidation. The heat used in

roasting can cause the flavonoid compounds to be oxidized or evaporated. In other words, these compounds are changed, and their quantity is reduced. Flavonoids are a group of polyphenol compounds, but not all polyphenol groups are flavonoids. Therefore, the phenolic changes do not always affect the flavonoids, but the changes in the flavonoids are affected by the phenolics (Mutha *et al*., 2021).

Caffeine Content

Caffeine is a group of alkaloids derived from xanthines found in almost all coffee types. Table 4 presents the total caffeine contents in original and fermented Liberica coffee. The OLC sample had a caffeine content of 0.858%, and the FLC sample was 0.821%. It shows a decrease in caffeine levels in fermented Liberica coffee. Nevertheless, the caffeine content obtained met the standard values of the standard ground coffee by the Indonesian National Standard (SNI 01-3542-2004), namely 0.45% – 2%.

Table 4. Caffeine contents in fermented and original Liberica coffee

Samples	Caffeine Content (%)		Average ± SD (%)
	I	II	
OLC	0.857	0.859	0.858 ± 0.01
FLC	0.807	0.810	0.821 ± 0.01

Note: OLC (Original Liberica Coffee) and FLC (Fermented Liberica Coffee)

The caffeine in coffee is broken down into ester compounds through fermentation using *B. subtilis* bacteria. Caffeine belongs to a class of alkaloid compounds. It is broken down into esters such as chlorogenic acid through an esterification process to reduce coffee's caffeine content (decaffeinated). The decrease in caffeine levels is due to the long fermentation. Microbial activity can produce various enzymes that degrade multiple compounds in coffee beans during fermentation (Haile and Kang, 2019). Bacteria absorb caffeine because the structure of caffeine is similar to that of purine molecules. Several bacteria, such as Pseudomonas, Rhodococcus, Brevibacterium, and Bacillus, can degrade caffeine. They degrade caffeine to obtain a carbon source by demethylating the caffeine. Some bacteria have also been reported to be able to degrade caffeine into parazanthine, 7-methylxanthine, and 1-methylxanthine. Furthermore, they can degrade up to 96% of caffeine if the fermentation is continuously done (Purwoko *et al*., 2023).

B. subtilis is a proteolytic bacterium that produces high levels of proteolytic enzymes (Ibrahim *et al*., 2014). Prior studies stated that the activity of proteolytic bacteria producing high enough protease enzymes can reduce the amount of caffeine in coffee beans. Coffee with low caffeine content increases free amino acids from protein decomposition (Farida *et al*., 2013).

Chlorogenic Acid Content

The chlorogenic acid compound is crucial in determining the quality, taste, and aroma of coffee beans. This research measured the percentage of chlorogenic acid in the prepared coffee, as seen in Table 5.

Table 5. Chlorogenic acid contents in fermented and original Liberica coffee

Samples	Chlorogenic Acid Content (%)		Average± SD (%)
	I	II	
OLC	2.988	2.981	2.984 ± 0.05
FLC	2.966	2.960	2.965 ± 0.04

Note: OLC (Original Liberica Coffee) and FLC (Fermented Liberica Coffee)

The levels of chlorogenic acid in the OLC and FLC samples were 2.981% and 2.960%, respectively. The decreased chlorogenic acid in the FLC sample was influenced by the addition of bacteria during fermentation. During the process, *B. subtilis* breaks down ester compounds into several derivative compounds with acid structures, such as quinic acid and cinnamic acid. After decomposing chlorogenic acid into other organic compounds, it dissolves in the fermentation medium. The longer dissolution process results in more chlorogenic acid dissolving in the fermentation medium. Chlorogenic acid is hydrolyzed into free caffeic acid and quinic acid due to the influence of *B. subtilis* bacteria.

Proximate Analysis

The proximate analysis included moisture, ash, protein, fat, and carbohydrate contents. The results are presented in Table 6.

Table 6. Moisture, ash, protein, fat, and carbohydrate contents in fermented and original Liberica coffee

Parameters	Sample	
	OLC	FLC
Moisture (%)	4.30	3.23
Ash (%)	4.17	3.46
Protein (%)	17.61	14.74
Fat (%)	10.71	9.28
Carbohydrate (%)	5.94	3.67

Note: OLC (Original Liberica Coffee) and FLC (Fermented Liberica Coffee)

Moisture Content

Moisture content is another aspect used to indicate the quality of coffee beans. It contributes to the growth of mold and the formation of the aroma and taste of coffee products. Excessive water content can cause mold to form on coffee products during storage. Therefore, the water content is one component that is constantly tested on coffee products. Based on the results at Table 6, the water content in the two samples complied with the requirements of the standard ground coffee by SNI 01-3542-2004, which is a maximum of 7%.

Further, the water content in FLC was lower than that of OLC. The result was affected by the fermentation process. During the fermentation process, the temperature increases due to bacteria, so the pores of the coffee beans open, and evaporation occurs. Fermentation also changes the hygroscopic sugar compounds in the mucilage from coffee beans into alcohol (Murthy and Naidu, 2011). As a result, the dried coffee beans processed into powder experience a decrease in water content as the number of inoculations increases. The results of this research were similar to other research, revealing that the coffee fermentation process by bacteria causes the coffee to absorb large amounts of water so that the moisture content in the fermented coffee beans increases (Nizori *et al*., 2021).

Ash Content

Ash is an inorganic residue from burning a portion of food at a temperature of 550 °C Ash content shows the total minerals contained in a food. The ash contents in the original and fermented Liberica coffee were 4.20% and 3.46%, respectively, as shown in Table 6. It shows that the ash content in the two samples met the requirements of standard ground coffee by SNI 01-3542-2004, which should be less than 5%. Further, the OLC sample had a higher ash content than the FLC sample. A high ash content indicates a high mineral content. Dirt and the remaining epidermis can also affect the ash content contained in coffee beans. Contrarily, the decrease in ash content during fermentation is caused by bacteria using minerals, vitamins, and sugars in the material for growth as an energy source. Other studies also reported that the fermentation duration with *B. subtilis* significantly affected the ash content in the coffee. The longer fermentation time resulted in the lower ash content. It is related to the dissolved compounds contained in coffee beans during fermentation (Sipayung *et al*., 2019).

Protein Content

Based on Table 6, the OLC sample had a higher protein content than the FLC sample, namely 17.61% and 14.74%, respectively. This is because, in fermented coffee, there is competition between bacteria using nutrients to produce metabolites. According to the previous study, a decrease in protein levels during fermentation is caused by *B. subtilis* becoming proteolytic (Balachandran *et al*., 2021). During fermentation, *B. subtilis* produces protease enzymes that degrade proteins into amino acids, peptides, and ammonia (Chukeatirote, 2015). Besides, during fermentation with *B. subtilis*, several proteins are used as a nitrogen source for their growth so that protein decreases. The protein hydrolysis reaction breaks down peptides to form amino acids and reduces the bitter taste and acidity of the coffee drinks. The breakdown of protein causes a decrease in the caffeine content of coffee and an increase in free amino acids (Alamri *et al*., 2022). A study by Wibowo *et al*. (2021) also reported that the protein content in fermented Liberica coffee ranged from 12.89 to 15.85%.

Fat Content

The fat component in coffee is one of the chemical compounds that contributes to and forms the different taste of coffee. An increase in free fatty acids during storage will cause rancidity in the coffee grounds, affecting the taste and reducing the quality of the coffee grounds (Hayati *et al*., 2012). Based on Table 6, the fat content in coffee beans decreased due to the fermentation, whereas the values of the OLC and FLC samples were 10.17% and 9.28%, respectively. Previous studies reported that the fat content in coffee fermented for 12 hours ranged from 9.48% – 10.21% (Wibowo *et al*., 2021). Other research exposed that fermentation could reduce the fat content in coffee beans. Prior studies also noted that the fermentation duration of Luwak coffee could decrease fat content (Prakash *et al*., 2022). The decrease in fat content during fermentation is caused by *B. subtilis*, which is a lipolytic that can hydrolyze fat. Fermentation is the breakdown of proteins, fats, and carbohydrates by bacterial activity, so there are simple fractions (Uliyandari *et al*., 2021).

Carbohydrate Content

Carbohydrates are one of the components in coffee beans that determine the formation of the coffee aroma, primarily through the caramelization of sugar through the Maillard reaction. Table 6 shows that the carbohydrate content of fermented coffee decreased to 3.67%. Previous studies mentioned that one of the causes of the decrease in carbohydrate content is the breakdown of polysaccharides into monosaccharides (Su *et al*., 2023). Besides, the bacteria present during fermentation require energy from overhauling organic and non-organic compounds, mainly provided by carbohydrate compounds. The breakdown of carbohydrates causes the sweet taste characteristic of coffee beans to become glucose during fermentation, affecting the sweet taste (Redgwell and Fischer, 2006)

Antioxidant Activity

The antioxidant activity test of Liberica coffee was carried out using the 2,2-diphenyl-1-picrylhydrazyl (DPPH). The principle of measuring antioxidant activity quantitatively using the DPPH method is a change in the intensity of the purple color of DPPH in direct proportion to the DPPH solution concentration. DPPH free radicals with unpaired electrons produce a purple color. When the electrons are paired, the color will turn yellow. The change in the intensity of the purple color occurs due to the attenuation of free radical compounds produced by the reaction of the DPPH molecule with the hydrogen atoms released by the compound molecule. The reaction forms diphenylpicrylhydrazyl and causes the DPPH color to decay from purple to yellow. The antioxidant activities of the samples are displayed in Table 7.

Table 7. Antioxidant activities of fermented and original Liberica coffee

Samples	IC_{50} (ppm)
OLC	72.122
FLC	42.368

Note: OLC (Original Liberica Coffee) and FLC (Fermented Liberica Coffee)

The IC_{50} value is defined as the concentration of the tested compound, which can reduce free radicals by 50%. The smaller IC_{50} value means the higher antioxidant activity of a sample. A compound is said to have robust antioxidant activity if the IC_{50} value is less than 50 ppm. The strong group is between 50 – 100 ppm. The medium group is between 101 – 150 ppm. Meanwhile, the weak group is between 151 – 200 ppm. According to Table 7, the FLC sample was classified as a robust group with an IC_{50} value of 42.368. Meanwhile, the OLC sample was categorized as the strong group with an IC_{50} value of 72.122. The antioxidant activity of the fermented coffee beans was enhanced due to the free phenolic produced during the fermentation process. The higher phenolic content created induces higher antioxidant activity. Organic acids also influence the increased antioxidant activity in fermented coffee beans. Similar research applying yeast fermentation also reported that fermentation can improve the functional properties of coffee by increasing the IC_{50} value of its antioxidant activity (Kwak *et al*., 2018).

Volatile Compounds

In GC-MS, gas chromatography functions to separate the various molecules in the sample while the mass spectrometer detects each separated component molecule. The result of GC-MS analysis is a chromatogram where

each peak represents chemical components. Based on the results of the GC-MS analysis, a chromatogram was obtained for the OLC sample, which reported in Table 8

Table 8. Profile of OLC compounds from the GC-MS analysis.

Compound Peak	R. Time (Minutes)	Area (%)	Hit	Formula	Compound Name
1	22.308	7.98	1	$C_{39}H_{74}O_6$	Dodecanoic Acid, 1,2,3-Propanetriyl Ester
			2	$C_{27}H_{52}O_5$	Dodecanoic Acid, 1-(Hydroxymethyl)-1,2-Ethanediyl Ester
			3	$C_{57}H_{10}O_6$	Glyceryl Tridodecanoate
2	22.450	30.97	1	$C_{39}H_{74}O_6$	Dodecanoic Acid, 1,2,3-Propanetriyl Ester
			2	$C_{27}H_{52}O_5$	Dodecanoic Acid, 1-(Hydroxymethyl)-1,2-Ethanediyl Ester
			3	$C_{57}H_{10}O_6$	Glyceryl Tridodecanoate
3	22.625	10.30	1	$C_{49}H_{94}O_6$	Octadecanoic Acid,2,3-Bis[(1-Oxotetradecyl) Oxy] Propyl Ester
			2	$C_{57}H_{10}O_6$	Glyceryl Tridodecanoate
			3	$C_{27}H_{52}O_5$	Dodecanoic Acid, 1-(Hydroxymethyl)-1,2-Ethanediyl Ester
4	22.800	7.16	1	$C_{39}H_{74}O_6$	Dodecanoic Acid, 1,2,3-Propanetriyl Ester
			2	$C_{57}H_{10}O_6$	Glyceryl Tridodecanoate
			3	$C_{35}H_{66}O_5$	2-Lauro-1,3 Didecoin
5	22.875	5.79	1	$C_{39}H_{74}O_6$	Dodecanoic Acid, 1,2,3-Propanetriyl Ester
			2	$C_{57}H_{10}O_6$	Glyceryl Tridodecanoate
			3	$C_{27}H_{52}O_5$	Dodecanoic Acid, 1-(Hydroxymethyl)-1,2-Ethanediyl Ester
6	22.942	9.84	1	$C_{39}H_{74}O_6$	Dodecanoic Acid, 1,2,3-Propanetriyl Ester
			2	$C_{57}H_{10}O_6$	Glyceryl Tridodecanoate
			3	$C_{35}H_{66}O_5$	2-Lauro-1,3 Didecoin
7	23.092	15.82	1	$C_{39}H_{74}O_6$	Dodecanoic Acid, 1,2,3-Propanetriyl Ester
			2	$C_{57}H_{10}O_6$	Glyceryl Tridodecanoate
			3	$C_{26}H_{48}F_3NO_2$	Dodecanamide, N-Dodecyl-N-(Trifluoroacetyl)
8	23.150	8.41	1	$C_{39}H_{74}O_6$	Dodecanoic Acid, 1,2,3-Propanetriyl Ester
			2	$C_{57}H_{10}O_6$	Glyceryl Tridodecanoate
			3	$C_{26}H_{48}F_3NO_2$	Dodecanamide, N-Dodecyl-N-(Trifluoroacetyl)
9	23.283	2.35	1	$C_{39}H_{74}O_6$	Dodecanoic Acid, 1,2,3-Propanetriyl Ester
			2	$C_{47}H_{90}O_6$	Hexadecanoic Acid,2-[(1-Oxododecyl) Oxy]-1,3-Propanediyl Ester
			3	$C_{55}H_{106}O_6$	Eicosanoic acid, 2-[(1-oxohexadecyl)oxy]-1-[[(1-oxohexadecyl)oxy]methyl]ethyl ester
10	23.442	1.03	1	$C_{39}H_{74}O_6$	Dodecanoic Acid, 1,2,3-Propanetriyl Ester
			2	$C_{14}H_{26}O_2$	Dodecanis Acid, Ethenyl Ester
			3	$C_{17}H_{36}$	2,6,10-Trimethyltetradecane
			4	$C_{35}H_{66}O_5$	2-Lauro-1,3 Didecoin
11	23.542	0.35	1	$C_{39}H_{74}O_6$	Dodecanoic Acid, 1,2,3-Propanetriyl Ester
			2	$C_{14}H_{26}O_2$	Dodecanis Acid, Ethenyl Ester
			3	$C_{27}H_{52}O_5$	Dodecanoic Acid, 1-(Hydroxymethyl)-1,2-Ethanediyl Ester

Table 8 presents the results of the GC-MS analysis of the OLC sample. The result reveals 11 detected peaks with varying percentage areas (Table 8). These peaks indicated the type of Liberica coffee compound before it was fermented. The high or low concentration of the identified compounds is indicated by the high or low peak area (%). The higher peak denotes the higher concentration of the compound. Meanwhile, the lower peak means the lower concentration of the compound. Table 8 demonstrates the highest peaks representing three compounds, including Dodecanoic acid, 1,2,3-propanetriyl ester ($C_{39}H_{74}O_6$), Dodecanoic acid, 1-(hydroxymethyl)-1,2-

ethanediyl ester ($C_{27}H_{52}O_5$), Glyceryl tridodecanoate ($C_{57}H_{10}O_6$) with a retention time of 22,450 minutes and an area percentage of 30.97%.

The results of GC-MS analysis of the FLC sample are demonstrated in Table 9. From the FLC chromatogram, 19 peaks were detected, indicating the presence of 19 chemical compounds contained in Liberica coffee after fermentation. Before Liberica coffee was fermented, 11 peaks were generated. This means there were 8 peaks in addition, indicating an increase in the chemical components of Liberica coffee after fermentation. The chromatogram of the FLC exhibits three highest peaks, denoting three compounds of Dodecanoic acid,1,2,3-propanetriyl ester ($C_{39}H_{74}O_6$), Glyceryl tridodecanoate ($C_{57}H_{10}O_6$), Dodecanamide, N-Trifluoroacetyl-N-Dodecyldodecanamide ($C_{26}H_{48}F_3NO_2$) which appeared at a retention time of 23.792 minutes and an area percentage of 8.99%. The compound profiles resulting from the GC-MS analysis of FLC can be seen in Table 9.

Table 9. Profile of FLC compounds from the GC-MS analysis.

Compound Peak	R. Time (Minutes)	Area (%)	Hit	Formula	Compound Name
1	20.458	0.69	1	$C_{39}H_{74}O_6$	Dodecanoic acid, 1,2,3-propanetriyl ester
			2	$C_{57}H_{10}O_6$	Glyceryl Tridodecanoate
			3	$C_{27}H_{52}O_5$	Dodecanoic Acid, 1-(Hydroxymethyl)-1,2-Ethanediyl Ester
2	20.750	1.05	1	$C_{39}H_{74}O_6$	Dodecanoic Acid, 1,2,3-Propanetriyl Ester
			2	$C_{57}H_{10}O_6$	Glyceryl Tridodecanoate
			3	$C_{27}H_{52}O_5$	Dodecanoic Acid, 1-(Hydroxymethyl)-1,2-Ethanediyl Ester
3	20.808	0.83	1	$C_{39}H_{74}O_6$	Dodecanoic Acid, 1,2,3-Propanetriyl Ester
			2	$C_{57}H_{10}O_6$	Glyceryl Tridodecanoate
			3	$C_{27}H_{52}O_5$	Dodecanoic Acid, 1-(Hydroxymethyl)-1,2-Ethanediyl Ester
4	20.942	1.44	1	$C_{39}H_{74}O_6$	Dodecanoic Acid, 1,2,3-Propanetriyl Ester
			2	$C_{57}H_{10}O_6$	Glyceryl Tridodecanoate
			3	$C_{27}H_{52}O_5$	Dodecanoic Acid, 1-(Hydroxymethyl)-1,2-Ethanediyl Ester
5	21.050	2.33	1	$C_{39}H_{74}O_6$	Dodecanoic Acid, 1,2,3-Propanetriyl Ester
			2	$C_{57}H_{10}O_6$	Glyceryl Tridodecanoate
			3	$C_{27}H_{52}O_5$	Dodecanoic Acid, 1-(Hydroxymethyl)-1,2-Ethanediyl Ester
6	21.242	2.95	1	$C_{39}H_{74}O_6$	Dodecanoic Acid, 1,2,3-Propanetriyl Ester
			2	$C_{57}H_{10}O_6$	Glyceryl Tridodecanoate
			3	$C_{27}H_{52}O_5$	Dodecanoic Acid, 1-(Hydroxymethyl)-1,2-Ethanediyl Ester
7	21.375	2.08	1	$C_{39}H_{74}O_6$	Dodecanoic Acid, 1,2,3-Propanetriyl Ester
			2	$C_{27}H_{52}O_5$	Dodecanoic Acid, 1-(Hydroxymethyl)-1,2-Ethanediyl Ester
			3	$C_{14}H_{26}O_2$	Dodecanoic Acid, Ethenyl Ester
8	21.467	3.66	1	$C_{39}H_{74}O_6$	Dodecanoic Acid, 1,2,3-Propanetriyl Ester
			2	$C_{27}H_{52}O_5$	Dodecanoic Acid, 1-(Hydroxymethyl)-1,2-Ethanediyl Ester
			3	$C_{57}H_{10}O_6$	Glyceryl Tridodecanoate
9	21.542	5.38	1	$C_{39}H_{74}O_6$	Dodecanoic Acid, 1,2,3-Propanetriyl Ester
			2	$C_{57}H_{10}O_6$	Glyceryl Tridodecanoate
			3	$C_{27}H_{52}O_5$	Dodecanoic Acid, 1-(Hydroxymethyl)-1,2-Ethanediyl Ester
10	21.683	6.48	1	$C_{39}H_{74}O_6$	Dodecanoic Acid, 1,2,3-Propanetriyl Ester
			2	$C_{57}H_{10}O_6$	Glyceryl Tridodecanoate
			3	$C_{27}H_{52}O_5$	Dodecanoic Acid, 1-(Hydroxymethyl)-1,2-Ethanediyl Ester
11	21.850	5.50	1	$C_{39}H_{74}O_6$	Dodecanoic Acid, 1,2,3-Propanetriyl Ester
			2	$C_{57}H_{10}O_6$	Glyceryl Tridodecanoate
			3	$C_{51}H_{98}O_6$	Octadecanoic Acid, 3-[(1-Oxododecyl)Oxy]-1,2-Propanediyl Ester
12	21.975	3.71	1	$C_{19}H_{40}$	Nonadecane

Compound Peak	R. Time (Minutes)	Area (%)	Hit	Formula	Compound Name
			2	$C_{39}H_{74}O_6$	Dodecanoic Acid, 1,2,3-Propanetriyl Ester
			3	$C_{20}H_{42}$	Eicosane
				$C_{23}H_{48}$	Tricosane
13	22.150	7.39	1	$C_{39}H_{74}O_6$	Dodecanoic Acid, 1,2,3-Propanetriyl Ester
			2	$C_{57}H_{10}O_6$	Glyceryl Tridodecanoate
			3	$C_{47}H_{90}O_6$	Hexadecanoic Acid, 2-[(1-Oxododecyl)Oxy]-1,3-Propanediyl Ester
14	22.275	3.43	1	$C_{39}H_{74}O_6$	Dodecanoic Acid, 1,2,3-Propanetriyl Ester
			2	$C_{47}H_{90}O_6$	Hexadecanoic Acid, 2-[(1-Oxododecyl)Oxy]-1,3-Propanediyl Ester
			3	$C_{57}H_{10}O_6$	Glyceryl Tridodecanoate
15	23.383	3.13	1	$C_{39}H_{74}O_6$	Dodecanoic Acid, 1,2,3-Propanetriyl Ester
			2	$C_{57}H_{10}O_6$	Glyceryl Tridodecanoate
			3	$C_{27}H_{52}O_5$	Dodecanoic Acid, 1-(Hydroxymethyl)-1,2-Ethanediyl Ester
16	23.525	8.20	1	$C_{39}H_{74}O_6$	Dodecanoic Acid, 1,2,3-Propanetriyl Ester
			2	$C_{57}H_{10}O_6$	Glyceryl Tridodecanoate
			3	$C_{35}H_{66}$	2-Lauro-1,3-Didecoin
17	23.658	13.83	1	$C_{39}H_{74}O_6$	Dodecanoic Acid, 1,2,3-Propanetriyl Ester
			2	$C_{57}H_{10}O_6$	Glyceryl Tridodecanoate
			3	C35H66	2-Lauro-1,3-Didecoin
18	23.792	18.96	1	$C_{39}H_{74}O_6$	Dodecanoic Acid, 1,2,3-Propanetriyl Ester
			2	$C_{57}H_{10}O_6$	Glyceryl Tridodecanoate
			3	$C_{26}H_{48}F_3NO_2$	N-Trifluoroacetyl-N-Dodecyldodecanamide
19	23.900	8.99	1	$C_{39}H_{74}O_6$	Dodecanoic Acid, 1,2,3-Propanetriyl Ester
			2	$C_{57}H_{10}O_6$	Glyceryl Tridodecanoate
			3	$C_{14}H_{26}O_2$	Dodecanoic Acid, Ethenyl Ester

According to the results, the fermentation using *B. subtilis* bacteria succeeded in adding new compounds to the fermented Liberica coffee. Similar research also claimed that fermentation could produce volatile compounds, including acetaldehyde, ethanol, ethyl acetate, isoamyl acetate, caramel, and fruit flavors (Lee *et al*., 2015).

CONCLUSION

The research results showed that the total phenolics, flavonoids, antioxidants, and volatile compounds of Liberica coffee fermented using *B. subtilis* were higher than those of the original Liberica coffee. Meanwhile, caffeine and chlorogenic acid contents in fermented Liberica coffee using *B. subtilis* were lower than in the original Liberica coffee. Thus, overall, the physicochemical and sensory properties of the Liberica coffee fermented using *B. subtilis* bacteria have been improved.

CONFLICT OF INTEREST

There is no conflict of interest in this article

AUTHOR CONTRIBUTION

ILT: Project Administration, Performed the experiment, Data Analysis, Methodology, Manuscript Drafting; EA: Experiment conduction, Statistical analysis; HH: Statistical analysis; ML: Supervision, Manuscript Drafting; SS: Conceptualization, Supervision, Manuscript Review, Funding Acquisition.

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