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The quality of robusta coffee (*Coffea canephora*) with the addition of *Leuconostoc mesenteroides* during wet fermentation

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Abstract. *Rosaliana F, Swasti YR, Purwijantiningsih E. 2025. The quality of Robusta coffee* (Coffea canephora) *with the addition of* Leuconostoc mesenteroides *during wet fermentation. Biodiversitas 26: 3334-3342.* Coffee consumption is inseparable from the health benefits of antioxidants in coffee beans. However, coffee contains caffeine, which affects the nervous system, kidneys, and blood vessels. Caffeine content is usually reduced using organic solvents and physical treatment. However, caffeine reduction using microorganisms, such as Lactic Acid Bacteria (LAB), is underexplored. This research aimed to improve the quality of robusta coffee by increasing its antioxidant activity and reducing caffeine content by adding *Leuconostoc mesenteroides* during wet fermentation. *L. mesenteroides* is a proteolytic bacterium that can degrade caffeine in coffee beans and enhance antioxidant activity. Robusta coffee has a higher caffeine content than Arabica. Therefore, the caffeine content needs to be reduced. The caffeine content was analysed using the HPLC method, and antioxidant activity was conducted using the DPPH method. The experimental design in this study was a completely randomized factorial design with two factors: fermentation time and the addition of *L. mesenteroides*. Each treatment has 3 replications. The data were analyzed using Analysis of Variance (ANOVA). The results show that the average caffeine content of espresso robusta green coffee beans was 1.26-2.19 mg/mL, while that of roasted coffee beans was 2.05-3.04%. Adding *L. mesenteroides* reduces the caffeine content of coffee beans by 13.34%. Espresso with green coffee beans has the average antioxidant activity ranging from 17.57±0.08%-23.32±0.08%, and espresso with roasted coffee beans ranges from 80.97±0.21%-84.87±0.08%; *L. mesenteroides* increases the antioxidant activity by 1.06%. In addition, the average moisture content of robusta green coffee beans fermented at 6, 12, and 18 hours with natural microbes and the addition of *L. mesenteroides* reached a moisture content of 9.82±0.10%-10.83±0.32%, while the roasted coffee beans ranged from 3.33±0.23%-3.80±0.16%. The average protein content of green coffee beans ranged from 10.51±0.76% to 13.28±0.67%, and roasted coffee beans from 11.38±0.88% to 12.55±0.50%. The total phenolic compound of green coffee beans ranged from 18.14±0.31 to 31.32±0.14 mg GAE/g, while the roasted beans reached 85.37±0.30 to 123.87±1.01 mg GAE/g. Adding *L. mesenteroides* reduces pH of green and roasted coffee beans. Testing other LABs on coffee beans can further explore these potential health benefits.

Keywords: Antioxidant, caffeine, coffee, fermentation, *Leuconostoc mesenteroides*

INTRODUCTION

Post-harvest coffee processing is generally conducted through either dry or wet fermentation (Galanakis 2017). Fermentation breaks down carbohydrates and amino acids, removing the mucus layer that sticks to the coffee beans. Dry fermentation involves drying all parts of the coffee fruit under the sun to obtain a water content of ≤12.5%. Then, the fruit skin, flesh, and seeds are separated with a peeling machine. Wet fermentation utilizes a tool to separate the skin, fruit, and coffee beans. Then, the coffee beans are soaked in water for 24 hours and dried under the sun to obtain a water content of ≤12.5%. After that, peeling separates the skin from the coffee beans (De Bruyn et al. 2016). Fermentation can affect a coffee's taste, aroma, and chemical composition. The wet fermentation process of coffee beans increases antioxidant activity. It is likely due to the presence of spontaneous LAB that can produce the phenolic compound through an enzymatic reaction (Fessard et al. 2017); specifically feruloyl-esterase with the cell wall as substrate (Sidoryk et al. 2018) and tannase enzymes with tannin as a substrate (Kostinek et al. 2007).

Caffeine (1,3,7-trimethylxanthine) in coffee is an alkaloid that occurs in varying amounts in brewed coffee (Higdon and Frei 2006). As recommended by Health Canada and the European Food Safety Authority, the daily caffeine intake for adults and children is 400 mg/day and 2.5-3.0 mg/kgBW/day, respectively (Verster and Koenig 2018). The caffeine content of coffee drinks depends on the type of coffee beans, the brewing strength, and the roasting process. Generally, coffee brewed similarly has a caffeine content ranging from 130 to 282 mg/240 mL (Higdon and Frei 2006). Arabica coffee contains caffeine ranging from 36-112 mg/100 mL, whereas robusta has a caffeine content ranging from 56 to 203 mg/100 mL (Oestreich-Janzen 2010).

The microbiota involved in coffee bean fermentation are bacteria, yeast, and filamentous fungi; bacteria are the most dominant (Shen et al. 2025). Some LABs (*Lactobacillus* most dominant compared to *Leuconostoc*) are cellulolytic bacteria that can hydrolyze complex cellulose, lignin, and hemicellulose into sugar, simple oligosaccharides (water-soluble carbohydrates), and lactic acid (Utama et al. 2018; Liu et al. 2020; Liao et al. 2022; Li et al. 2023). LABs also contain feruloyl-esterase, which can release antioxidant compounds from cell walls (Fessard et al. 2017). Additionally,

some LABs exhibit pectinolytic activity, which enables them to degrade pectin in the mucus layer of coffee beans (Saanu 2017; Reichembach and de Oliveira Petkowicz 2020). One species of LAB with pectinolytic activity is *L. mesenteroides*, which can affect the chemical quality and accelerate fermentation. *Leuconostoc mesenteroides* (8.54 log10 cells/mL) is the most dominant LAB and it persists until the end of wet fermentation (de Jesus Cassimiro et al. 2023). *L. mesenteroides* possesses proteolytic enzyme activity that can break down caffeine and produce 3,7-dimethyl xanthine, 7-methyl xanthine, and xanthine (Liu 2016). *L. mesenteroides* can also degrade caffeine by more than 89% and then produce paraxanthine during the wet fermentation of robusta coffee (Purwoko et al. 2022, 2023). Paraxanthine is considered safer than caffeine because it does not exhibit toxicological effects on rats (Szlapinski et al. 2023). Free caffeine can diffuse through the cell walls of coffee beans, allowing it to dissolve in water (Gokulakrishnan et al. 2005). Additionally, *L. mesenteroides* can degrade chlorogenic acid, an antioxidant, during the wet fermentation of robusta coffee into caffeic acid, which also exhibits antioxidant activity (Liang and Kitts 2016; Purwoko et al. 2022; Yang et al. 2023). It also has feruloyl-esterase, which increases antioxidant content (Fessard et al. 2017. *L. mesenteroides* is the dominant LAB in the wet fermentation of coffee (Elhalis et al. 2020). The density of *L. mesenteroides* remains stable during wet coffee fermentation until 72 h. Its metabolic process produces organic acids (acetic, citric, lactic, malic, and succinic acid). The highest organic acid is malic acid (de Jesus Cassimiro et al. 2023), which exhibits antioxidant activity by reducing Malondialdehyde (MDA) content (Mousavi et al. 2022). Adding *L. mesenteroides* to wet coffee fermentation produces a dominant dark chocolate-like effect among its sensory characteristics (de Jesus Cassimiro et al. 2023).

This research aimed to determine the chemical quality of espresso with robusta green coffee beans and roasted coffee beans regarding moisture, ash, fat, protein, carbohydrate, pH, Total Phenolic Content (TPC), antioxidant activity, caffeine content, total plate count, and Total Yeast Mold Count (TYMC) with various times of wet fermentation and the addition of *L. mesenteroides*.

MATERIALS AND METHODS

Material

The primary material for the study is robusta coffee beans taken from the slope of Merapi Volcano in Kepuharjo, Cangkringan, Sleman, Special Region of Yogyakarta, Indonesia. The other materials are LAB *L. mesenteroides* strain KIBGE-IB22 (JQ 6583451), aquadest, concentrated H_2SO_4 (Mallinckrodt, Kentucky, USA), Catalyze N, hydrocloric acid (Merck, Damstadt, Germany), NaOH (Merck, Darmstadt, Germany), De Man Ragosa and Shape Agar (Oxoid, Basingstoke, United Kingdom), De Man Ragosa and Sharpe Broth (Oxoid, Basingstoke, United Kingdom), Buffer Peptone Water (Oxoid, Basingstoke, United Kingdom), Plate Count Agar (Oxoid, Basingstoke, United Kingdom), Potato Dextrose Agar (Oxoid, Basingstoke, United Kingdom), gallic acid (Sigma-Aldrich St. Louis, MO, USA), Folin-Ciocalteu reagent (Sigma-Aldrich St. Louis, MO, USA) with ratio 1:10, Na_2CO_3 7.5 % (b/v), DPPH (Aldrich, Germany), methanol (Sigma-Aldrich Damstadt, Germany), ethanol (Merck, Darmstadt, Germany), ascorbic acid (Sigma-Aldrich, Steinheim, Germany), borate acid (Sigma Aldrich), N-hexane (J.T. Baker USA), and standard caffeine (Steinheim, Germany).

Procedures

Reculturing isolate L. mesenteroides

One ose of the LAB commercial culture *L. mesenteroides* strain KIBGE-IB22 (JQ 6583451) was taken and inoculated by streak plate methods in De Man Ragosa and Sharpe Agar (MRSA) and then incubated for 24 hours at room temperature (25-30°C). LAB *L. mesenteroides* strain KIBGE-IB22 (JQ 6583451) was inoculated into De Man Ragosa and Sharpe Broth (MRSB) medium in the test tube and incubated for 24 hours at room temperature (Su et al. 2024).

Viability L. mesenteroides *strain KIBGE-IB22 (JQ 6583451)*

Culture LAB of *L. mesenteroides* strain KIBGE-IB22 (JQ 6583451) (1 mL) was dissolved in 9 mL of Buffer Peptone Water (BPW) and labeled as a dilution of 10^{-1}. The serial dilution was performed using the same procedure until the 10^{-8} dilution series (Figure 1). After serial dilution, the viability of LAB was assessed by homogenizing each dilution with MRSA using the pour plate method and incubating for 24 hours at 37°C (Ibrahim et al. 2023). The viability formula was used to calculate the culture of LAB as follows:

$$N = \frac{\Sigma C}{[(1 \times n1) + (0.1 \times n2)] \times d}$$

Where :

N : Number of colonies (CFU/mg)
ΣC : The sum of colonies in each petri dish
n1 : The number of colonies in the first dilution
n2 : The number of colonies in the subsequent dilution
d : The first dilution that meets the requirements

Wet fermentation of robusta coffee bean

Three hundred grams of robusta green bean (*Coffea canephora*) were added to 450 mL of distilled water. Then, they were inoculated with 10 mL of a starter culture of *L. mesenteroides* at a concentration of 1×10^7 CFU/mL. Then, it was incubated for 6, 12, and 18 hours in an open container (Kwak et al. 2018).

Roasting process

Green coffee is roasted to a medium level (198-200°C for 10-15 minutes) using a Vina Nha Trang (VNT) coffee roaster.

Extraction of robusta coffee

Eight grams of ground coffee beans are extracted using the Staresso Espresso Maker tool with 60 mL of water at 95°C for 30 seconds and a pressure of 9 bar.

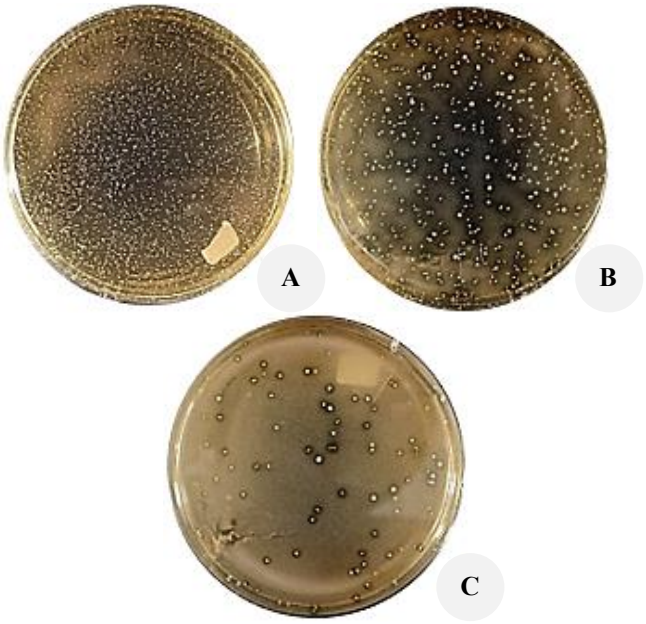

Figure 1. Viability of *Leuconostoc mesenteroides* by MRSA. A. 10^{-5}, B. 10^{-6}, C. 10^{-7}

Proximate test

The proximate test involves analyzing the moisture, ash, fat, protein, and carbohydrate content of both green and roasted coffee beans (Latimer 2023). Moisture content was measured using the gravimetry method with a moisture analyzer (Phoenix Instrument). Ash content was analyzed using gravimetry by burning samples at 550°C for 8 hours in a furnace (Onetech F-1400). Fat content was measured using the Soxhlet method for 4 hours, and n-hexane was used as the solvent. Protein content was analyzed using the Micro-Kjeldahl method, which measures the amount of nitrogen in the sample and then multiplies it by 6.25. Carbohydrate content was calculated using the carbohydrates by-difference method.

Degree of acidity

The degree of acidity is measured using a pH meter (SAFESEED Digital LCD Pocket Pen) and measured 3 times (Latimer 2023).

Determination of Total Phenolic Content (TPC)

TPC was measured using the Folin-Ciocalteu method. A standard curve of gallic acid was established using concentrations of 10, 20, 30, 40, and 50 ppm. 1 mL of each dilution series was mixed with 5 mL of Folin-Ciocalteu reagent (1:10) and allowed to stand for 5 minutes. Then, the solution is added with 4 mL of 7.5% Na_2CO_3 solution and incubated for 1 hour (Swasti et al. 2024). The absorbance solution was measured using an Ultraviolet-Visible (UV-VIS) Spectrophotometer (Genesys 10S) at 760 nm with the equation of linear regression as follows: Y (Dependent variable) = a + b.X (Independent Variable). The same procedure was performed on a sample of coffee espresso with a dilution of 10^{-2} for each type of espresso and repeated 3 times. The formula of the TPC was as follows:

$$\text{TPC} = \frac{\text{N. Phenolic (mg)} \times \text{extract volume (mL)}}{1000\ (\text{mL}) \times \text{weight of the sample (mg)} \times \text{dilution factor } (10^{-2})}$$

Determination of antioxidant activity with radical 2,2-diphenyl-1-picrylhydrazyl

Four milligrams of radical 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical were dissolved in 20 mL of 70% ethanol. Then, the absorbance of the DPPH stock was measured at 517 nm with an absorbance of 0.2-0.8 A. Next, 4 mL of the DPPH solution was added to 0.24 mL of 70% ethanol. Then, it was incubated in a dark room for 30 minutes, and the absorbance of the blank solution was measured at 517 nm. The sample of espresso robusta coffee was diluted to 10^{-2} with 70% ethanol. Then, 0.24 mL of the sample was taken from each dilution of the espresso sample and mixed with 4 mL of the DPPH stock solution. The solution was homogenized with a vortex and incubated at room temperature for 30 minutes. The absorbance was measured using a UV-VIS spectrophotometer (Genesys) at 517 nm (Hasanah et al. 2017). The assessment of antioxidant activity was repeated 3 times. The antioxidant value was calculated by dividing the absorbance difference of the blank from the sample by the absorbance of the blank and then multiplying by 100%. The formula is below:

$$\%\ \text{DPPH} = \frac{\text{Blank absorbance} - \text{Sample absorbance}}{\text{Blank absorbance}} \times 100\%$$

Determination of caffeine content using HPLC

Four milligrams of caffeine were diluted with 5 mL of aquabidest as a standard. A standard curve was constructed using concentrations of 100, 200, 300, 400, and 500 ppm. A 25 µL solution was injected into the columns with the mobile phase of aquabidest and methanol (75:25) at 272 nm. The green and roasted espresso coffee was centrifuged at 14,000 rpm and filtered using Whatman 42. One hundred µL of the espresso was added with 0.4 mL of aquabidest, and 25 µL was injected into an HPLC column at 272 nm (Naegele 2016). The caffeine content of the sample is calculated by interpolating the caffeine standard curve regression line equation and chromatogram graphs using SigmaPlot 14.0.

Microbiology tests of total plate count and Total Yeast and Mold Count (TYMC)

One gram of coffee powder was dissolved in 9 mL of aquadest, homogenized, and labeled as a10^{-1}dilution. The serial dilution was carried out using the same procedure until the 10^{-5} dilution series (Sari et al. 2025). 1 mL of the sample was taken, and each dilution series was placed with Plate Count Agar (PCA) medium using the pour plate method. Then, the total plate count was calculated. The Total Yeast and Mold Count (TYMC) test was performed as follows: 0.1 mL of the sample was taken and inoculated with a spread plate method on a solid Potato Dextrose Agar (PDA) medium. The total plate count and TYMC were calculated based on the dilution level and the number of colonies using the standard plate count method (Latimer 2023).

Sensory analysis using the Hedonic Test according to the Specialty Coffee Association of America

A total of 15 professional baristas became the sensory panelists for this study. Each panelist evaluates coffee samples for color, taste, aroma, and mouth feel using a 6-point organoleptic scale. A ranking test was carried out for overall preference assessment.

Data analysis

The data were analyzed using Analysis of Variance (ANOVA) to determine significant differences between treatments. The analysis was followed by the Duncan Multiple Range Test (DMRT) at a 95% confidence level. The data analysis was conducted using SPSS version 25 (IBM Corp., Chicago, USA) (George and Mallery 2018).

RESULTS AND DISCUSSION

The moisture content of the green and roasted coffee beans

The average moisture content of green coffee beans fermented in 6, 12, and 18 hours with natural microbes and the addition of *L. mesenteroides* ranged from 9.82±0.10% to 10.83±0.32% (Table 1), while roasted coffee beans had a moisture content of 3.33±0.23% to 3.80±0.16% (Table 2). These results meet the Indonesian National Standard for green coffee beans (01-2907-2008) and roasted beans (01-3542-2004), which require levels of less than 12.5% and less than 5%, respectively. Adding *L. mesenteroides* and extending the fermentation time decreases the moisture content of both green and roasted coffee beans, as *L. mesenteroides* possesses pectinolytic enzyme activity (Liu 2016).

The ash content of green and roasted coffee beans

The ash content of robusta green coffee beans ranges from 4.57±0.06% to 5.07±0.38 % (Table 1), while roasted coffee beans have an ash content of 5.07±0.15% to 5.33±0.21% (Table 2). Previous research by Oliveira et al. (2006) showed that the ash content of green coffee beans ranged from 4.8 to 5.8%. The previous study by Preedy (2015) showed that the ash content of roasted coffee ranges from 4.6 to 5.6%. The findings of this study align with the results of a previous study. Differences in ash content may be influenced by the soil mineral content of coffee plantations (Morales-Ramos et al. 2020). *L. mesenteroides* treatment did not affect the ash content.

The fat content of the green and roasted coffee beans

The fat content of robusta green coffee beans ranged from 6.30±0.40% to 7.33±0.15% (Table 1), while roasted coffee beans ranged from 10.00±0.26% to 10.57±0.25% (Table 2). Adding *L. mesenteroides* increased the fat content of green coffee beans due to the proteolytic activity of LABs, which degraded protein and further opened pores on the coffee beans' surfaces (Liu 2016). The opening of the pores facilitates the extraction of fat content with n-hexane more efficiently (Hanif et al. 2019). However, the average fat content of treated roasted coffee is not significantly different from that of the untreated coffee. It is likely due to high temperatures and pressures during roasting, which triggers more effective extraction of lipid content from the coffee beans.

The protein content of green and roasted coffee beans

The average protein content of robusta green coffee beans ranged from 10.51±0.76% to 13.28±0.67% (Table 1), while the roasted coffee beans ranged from 11.38±0.88% to 12.55±0.50% (Table 2). Adding *L. mesenteroides* and increasing the fermentation time resulted in a reduction of the protein content in green robusta coffee beans due to the proteolytic activities of *L. mesenteroides* (Liu 2016). Nevertheless, the average protein content of treated roasted coffee is not significantly different from that of untreated coffee. It is likely due to the higher amount of carbohydrates in treated green coffee compared to untreated coffee (Table 1). The amount of carbohydrates interacting with amino acids is greater during roasting.

The carbohydrate content of green and roasted coffee beans

The carbohydrates of robusta green coffee beans ranged from 64.91±1.38% to 67.64±0.75% (Table 1), while the carbohydrates of roasted coffee beans ranged from 68.91±0.18% to 69.58±1.01% (Table 2). Adding *L. mesenteroides* increased the carbohydrate content of green coffee beans because the dry weight of coffee beans decreased. After moisture removal, the dry weight comprises several components, i.e., carbohydrates, protein, amino acids, minerals, and vitamins (Wibowo et al. 2023). The increase in carbohydrate content in green coffee resulting from the addition of *L. mesenteroides* is likely due to the decrease in protein content (Table 1). However, the carbohydrate content of treated roasted coffee is not significantly different from that of the untreated one because the concentrations of ash, protein, and fat showed no significant changes.

The acidity of espresso with green and roasted coffee beans

The pH value of espresso with green coffee beans ranged from 6.43±0.01 to 6.54±0.02, and the pH value of espresso with roasted beans ranged from 5.62±0.01 to 5.88±0.01 (Table 3). The addition of *L. mesenteroides* decreases pH because it produces acid metabolites. *L. mesenteroides* is a heterofermentative bacterium that can produce alcohol, lactic acid, and acetic acid in the final metabolism (Koduru et al. 2018). It also produces more citric and malic acid than wet fermentation without the addition of *L. mesenteroides* (de Jesus Cassimiro et al. 2023). The roasting process can degrade sucrose, polysaccharides, and other components of coffee beans, allowing them to form aliphatic acids such as acetic, glycolic, formic, and lactic acids, which can increase the acidity of espresso with roasted coffee beans (Diviš et al. 2019).

The Total Phenolic Content (TPC) of single-shot espresso from green and roasted coffee beans

The TPC of robusta green coffee beans ranged from 18.14±0.31 to 31.32±0.14 mg GAE/g, and the TPC of the roasted coffee beans ranged from 85.37±0.30 to 123.87±1.01

mg GAE/g (Table 3). Adding LAB *L. mesenteroides* increases the TPC of coffee beans because it has the feruloyl-esterase and tannase enzymes (Fessard et al. 2017). Feruloyl-esterase releases ferulic acid and hydroxycinnamates from cell walls (lignin, cellulose, and hemicellulose) (Tai et al. 2014), which have antioxidant activity (Fessard et al. 2017; Oliveira et al. 2019). Tannase enzyme activity (Kostinek et al. 2007) can hydrolyze the tannins of coffee beans into tannic acid, gallic acid, n-propyl gallic, and isoarnil gallic, which contribute to the TPC of coffee beans (Aguilar-Zarate et al. 2014). Recent research showed that the roasting process of coffee beans can increase the TPC (Mestanza et al. 2023). The total phenol content of roasted coffee is higher than that of green coffee due to the increase of several phenolic compounds during roasting, such as 3,4-Dihydroxybenzoic acid, (+)-catechin, syringic acid, rutin-trihydrate, trans-ferulic acid, and isorhamnetin. Additionally, phenolic compounds such as caffeic acid are formed during the roasting process (Alkaltham et al. 2020).

Table 1. The percentage of the chemical content of green coffee beans

Fermentation time microbia	6 Hour	12 Hour	18 Hour	Average
Water content				
Natural microbes	10.83±0.32^{a}	10.15±0.03^{a}	10.01±0.25^{a}	10.33±0.44^{A}
Natural microbes + *Leuconostoc mesenteroides*	10.58±0.48^{a}	10.08±0.07^{a}	9.82±0.10^{a}	10.16±0.39^{A}
Average	10.70±0.18^{X}	10.12±0.04^{Y}	9.92±0.13^{Y}	
Ash content				
Natural microbes	5.07±0.38^{a}	4.87±0.47^{a}	5.03±0.42^{a}	4.99±0.11^{A}
Natural microbes + *Leuconostoc mesenteroides*	4.57±0.06^{a}	4.97±0.31^{a}	4.70±0.20^{a}	4.74±0.20^{A}
Average	4.82±0.35^{X}	4.92±0.07^{X}	4.87± 0.23^{X}	
Fat content				
Natural microbes	6.30±0.40^{a}	6.90±0.26^{a}	6.93±0.25^{a}	6.71±0.36^{A}
Natural microbes + *Leuconostoc mesenteroides*	6.93±0.15^{a}	7.07±0.21^{a}	7.33±0.15^{a}	7.11±0.20^{B}
Average	6.62±0.45^{X}	6.98±0.12^{Y}	7.13±0.28^{Y}	
Protein content				
Natural microbes	13.28±0.67^{a}	12.11±0.67^{a}	11.53±0.25^{a}	12.30±0,89^{A}
Natural microbes + *Leuconostoc mesenteroides*	12.26±0.44^{a}	11.53±0.25^{a}	10.51±0.76^{a}	11.43±0,88^{B}
Average	12.77±0.72^{X}	11.82±0.41^{Y}	11.02±0.72^{Z}	
Carbohydrate content				
Natural microbes	64.91±1.38^{a}	65.97±0.84^{a}	66.50±0.38^{a}	65.69±0.98^{A}
Natural microbes + *Leuconostoc mesenteroides*	65.66±0.84^{a}	66.36±0.17^{a}	67.64±0.75^{a}	66.55±1.00^{B}
Average	65.29±0.53^{X}	66.17±0.28^{Y}	67.07±0.81^{Y}	

Note: Average values are expressed as means±s.d, values with the same letter within the same column and row are not significantly different based on DMRT at a 95% confidence interval (n = 3)

Table 2. The percentage of the chemical content of roasted coffee beans

Fermentation time microbia	6 Hour	12 Hour	18 Hour	Average
Water content				
Natural microbes	3.57±0.13^{a}	3.76±0.14^{a}	3.80±0.16^{a}	3.70±0.12^{A}
Natural microbes + *Leuconostoc mesenteroides*	3.34±0.07^{a}	3.44±0.07^{a}	3.33±0.23^{a}	3.37±0.06^{B}
Average	3.46±0.16^{X}	3.60±0.23^{X}	3.57±0.33^{X}	
Ash content				
Natural microbes	5.27±0.15^{a}	5.33±0.21^{a}	5.13±0.06^{a}	5.24±0.10^{A}
Natural microbes + *Leuconostoc mesenteroides*	5.07±0.15^{a}	5.23±0.15^{a}	5.13±0.06^{a}	5.14±0.08^{A}
Average	5.17±0.14^{X}	5.28±0.07^{X}	5.13±0.00^{X}	
Fat content				
Natural microbes	10.00±0.26^{a}	10.23±0.25^{a}	10.30±0.53^{a}	10.18±0.16^{A}
Natural microbes + *Leuconostoc mesenteroides*	10.00±0.44^{a}	10.30±0.20^{a}	10.57±0.25^{a}	10.29±0.29^{A}
Average	10.00±0.00^{X}	10.27±0.05^{X}	10.43±0.19^{X}	
Protein content				
Natural microbes	12.26±0.44^{a}	11.67±0.51^{a}	11.67±0.25^{a}	11.87±0.34^{A}
Natural microbes + *Leuconostoc mesenteroides*	12.55±0.50^{a}	11.97±0.51^{a}	11.38±0.88^{a}	11.97±0.59^{A}
Average	12.40±0.21^{X}	11.82±0.21^{XY}	11.53±0.21^{Y}	
Carbohydrate content				
Natural microbes	68.91±0.18^{a}	69.00±0.29^{a}	69.10±0.59^{a}	69.00±0.13^{A}
Natural microbes + *Leuconostoc mesenteroides*	69.04±1.05^{a}	69.06±0.62^{a}	69.58±1.01^{a}	69.23±0.31^{A}
Average	68.98±0.09^{X}	69.03±0.04^{X}	69.34±0.34^{X}	

Note: Average values are expressed as means±s.d, values with the same letter within the same column and row are not significantly different based on DMRT at a 95% confidence interval (n = 3)

Table 3. The degree of acidity (pH), total phenolic, antioxidant activity, and caffeine content

Fermentation	pH	Total phenolic (mg GAE g^{-1})	Antioxidant activity (% inhibition)	Caffeine (mg/mL)
Green bean (natural microbes)				
6 Hour	6.54±0.02^{a}	26.30±0.70^{d}	22.76±0.29^{a}	2.19±0.08^{c}
12 Hour	6.54±0^{a}	22.44±0.21^{c}	20.77 ±0.21^{b}	1.75±0.10^{b}
18 Hour	6.48±0.02^{b}	20.78±0.31^{b}	17.57±0.08^{c}	1.68±0.08^{b}
Green bean (natural microbes + *Leuconostoc mesenteroides*)				
6 Hour	6.46±0.01^{b}	31.32±0.14^{f}	23.32±0.08^{e}	1.86±0.24^{b}
12 Hour	6.46±0.02^{b}	29.27±0.20^{e}	22.95±0.28^{d}	1.69±0.06^{b}
18 Hour	6.43±0.02^{c}	18.14±0.27^{a}	22.39±0.14^{d}	1.26±0.11^{a}
Roasted bean (natural microbes)				
6 Hour	5.88±0.01^{d}	113.25±0.76^{d}	83.52±0.08^{c}	3.04±0.19^{c}
12 Hour	5.74±0.01^{c}	99.09±0.67^{c}	82.27±0.08^{d}	2.59±0.21^{b}
18 Hour	5.63±0.02^{a}	91.35±1.02^{b}	81.43±0.08^{a}	2.38±0.15^{ab}
Roasted bean (natural microbes + *Leuconostoc mesenteroides*)				
6 Hour	5.68±0.01^{b}	119.00±0.67^{e}	84.03±0.16^{e}	2.75±0.13^{bc}
12 Hour	5.67±0.01^{b}	123.87±1.01^{f}	84.87±0.08^{f}	2.16±0.29^{a}
18 Hour	5.62±0.01^{a}	85.37±0.38^{a}	80.97±0.21^{b}	2.05±0.21^{a}

Note: Values are expressed as means±s.d, values with the same letter within the same column are not significantly different based on DMRT at a 95% confidence interval (n = 3)

The antioxidant activity of espresso from green and roasted coffee beans

The espresso of robusta green coffee beans has antioxidant activity ranging from 17.57±0.08% to 23.32±0.08%, while roasted coffee beans range from 80.97±0.21% to 84.87±0.08% (Table 3, Figure 2). The treatment with *L. mesenteroides* increased antioxidant activity because it can release antioxidant compounds from lignin, hemicellulose, and cellulose to form hydroxycinnamic acids (ferulic acid, p-coumaric, and caffeic acid) (Sidoryk et al. 2018; Oliveira et al. 2019). These findings align with research by Tarigan et al. (2023), which showed that the addition of *L. mesenteroides* in the wet fermentation of Liberica coffee enhances antioxidant activity. Research by de Jesus Cassimiro et al. (2023) showed that the increase in antioxidant activity is attributed to the higher content of citric and malic acid. Adding *L. mesenteroides* to roasted robusta coffee increases the chlorogenic acid content. The roasting process increased the antioxidant activity due to the formation of melanoidin compounds from the Maillard reaction (Choi et al. 2018), which produces heterocyclic compounds. Heterocyclic compounds increased antioxidant activity due to hydroxyl groups in the para position (Nishiyama et al. 2003). Heterocyclic compounds have a ring structure containing non-carbon atoms such as oxygen, nitrogen, and sulfur (Martins et al. 2015). The highest antioxidant activity was achieved at 12 hours of fermentation with the addition of *L. mesenteroides*. This finding aligns with a study by Swasti et al. (2024). Previous findings have shown that *L. mesenteroides* grows more rapidly in 10-12 hour wet fermentations than in shorter fermentations, i.e., those less than 10 hours (Zhang et al. 2019).

The caffeine content of espresso with green and roasted coffee beans

The average caffeine contents of espresso made with robusta green coffee beans and roasted coffee beans were 1.26 to 2.19 mg/mL and 2.05 to 3.04%, respectively (Table 3; Figure 3; Figure 4). These findings follow the research conducted by Wei et al. (2012), which found that the caffeine content of robusta green beans ranged from 1.6% to 2.4% and 2% to 2.8% for robusta green beans and robusta roasted beans, respectively. These findings align with a recent study conducted by Tarigan et al. (2023), which found that adding *L. mesenteroides* in the wet fermentation of Liberica coffee reduced caffeine content. The caffeine content of roasted robusta coffee with *L. mesenteroides* addition is lower than that without *L. mesenteroides* addition (de Jesus Cassimiro et al. 2023). *L. mesenteroides* reduced the caffeine content due to its proteolytic ability, which degrades proteins (Liu 2016). Caffeine has a chemical structure similar to that of protein, especially protein peptide bonds. Caffeine (1,3,7-trimethyl xanthine) is degraded due to the demethylation process, which produces 3,7-dimethyl xanthine, 7-methyl xanthine, and xanthine (Gokulakrishnan et al. 2005). Nevertheless, the ability of *L. mesenteroides* in reducing caffeine is lower compared to *Lactiplantibacillus plantarum* (de Jesus Cassimiro et al. 2023).

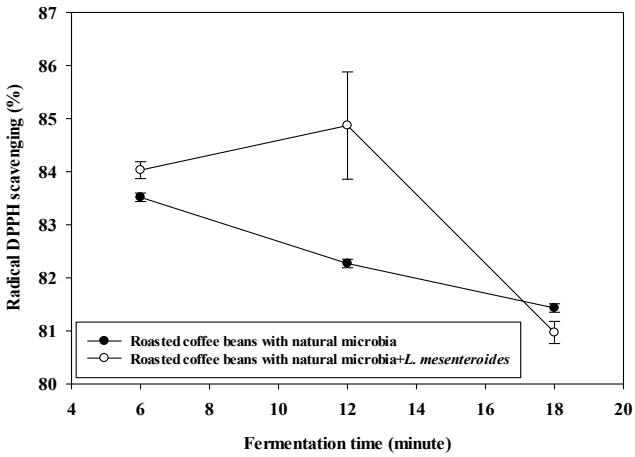

Figure 2. Radical DPPH scavenging of roasted coffee beans

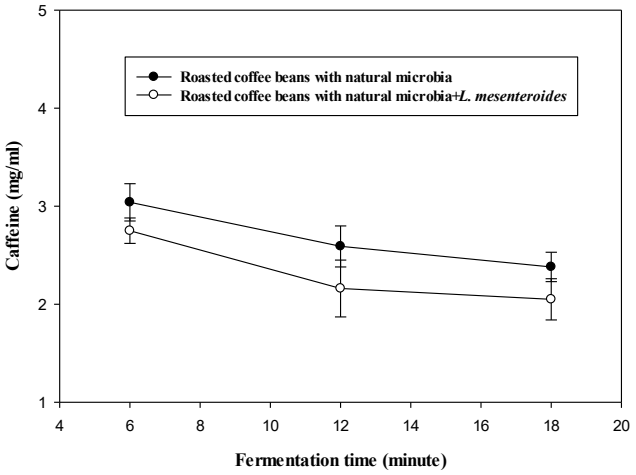

Figure 3. Caffeine of roasted coffee beans

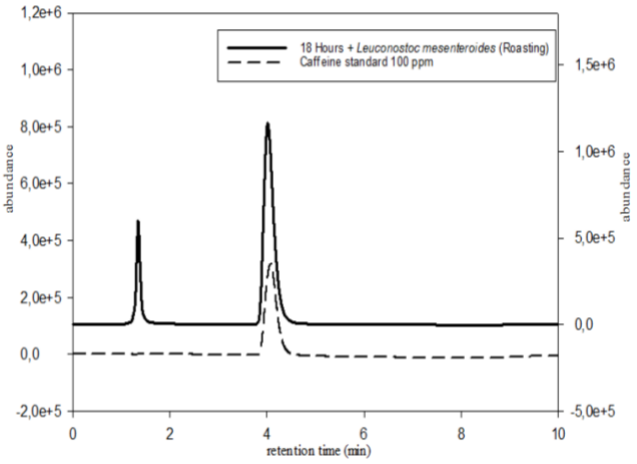

Figure 4. HPLC chromatogram of coffee espresso fermented for 18 hours with LAB

Table 5. Sensory properties of espresso roasted beans

Fermentation types	Fermentation time	Sensory properties				Average
		Color	Taste	Aroma	Mouthfeel	
Natural microbes	6 Hour	**4.20**	3.60	3.60	3.47	3.71
	12 Hour	3.93	3.33	3.07	3.60	3.48
	18 Hour	2.47	3.46	3.47	3.67	3.27
Natural microbes + *Leuconostoc mesenteroides*	6 Hour	3.87	**3.67**	3.33	**4.27**	**3.79**
	12 Hour	3.87	**3.67**	3.33	**4.27**	**3.79**
	18 Hour	2.73	3.53	**4.00**	2.68	3.24

Notes: 1: very much dislike, and 6: very like most

Table 4. Total plate count and Total Yeast Mold Count (TYMC) of roasted coffee beans

Fermentation types	Fermentation time	Total plate count (log CFU/g)	TYMC (log CFU/g)
Natural microbes	6 Hour	0.49±0 .85[a]	0.43±0.75[a]
	12 Hour	0±0[a]	0±0[a]
	18 Hour	1.99±0.24[a]	1.98±0.59[a]
Natural microbes + *Leuconostoc mesenteroides*	6 Hour	0±0[a]	0±0[a]
	12 Hour	0.33±0.58[a]	0±0[a]
	18 Hour	1.21±1.16[a]	1.26±0.24[a]

Note: Values are expressed as means±s.d, values with the same letter within the same row are not significantly different based on DMRT at a 95% confidence interval (n = 3)

Total plate count and Total Yeast Mold Count (TYMC) of roasted coffee beans

Table 4 shows the range of total plate count and TYMC of roasted coffee beans. The total plate count of roasted coffee beans reached 0 to 1.99±0.24 log CFU/g. The TYMC of roasted coffee beans ranged from 0 to 1.98±0.59 log CFU/g. This value falls within the range recommended by the Indonesian National Standard 01-3542-2004 regarding coffee powder, with total plate count and TYMC maximum at 6 log CFU/g and 4 log CFU/g, respectively. Adding *L. mesenteroides* decreases the total plate count and TYMC due to its antimicrobial components, including lactic acid, bacteriocins, and hydrogen peroxide (Rao et al. 2023). Lactic acid can diffuse into the cell membrane and acidify the cytoplasm (Zhang et al. 2021).

Sensory properties of espresso-roasted coffee beans

The color of espresso roasted coffee beans with *L. mesenteroides* treatment is less preferred due to the lower protein content in green coffee beans compared to the control (Table 5, Table 1), which reduces the intensity of the Maillard reaction (Choi et al. 2018). However, the taste, aroma, and mouthfeel of espresso roasted coffee beans with the addition of *L. mesenteroides* are more preferred compared to coffee without *L. mesenteroides* treatment due to the formation of acetic, citric, lactic, malic, and succinic acid during wet fermentation (Koduru et al. 2018; de Jesus Cassimiro et al. 2023). The addition of *L. mesenteroides* increases the intensity of dark chocolate perception of attributes and forms caramel perception of attributes (de Jesus Cassimiro et al. 2023) due to the higher carbohydrate content in green coffee beans compared to the control (Table 1). The samples with the addition of *L. mesenteroides* in fermentation times of 6 hours and 12 hours are the most liked products in terms of flavor because the longer fermentation time forms more acids (Koduru et al. 2018). This finding is in line with the previous study, which found that the addition of starter cultures during dry fermentation

of coffee cherries increased the sensory quality (Park et al. 2025).

In conclusion, protein, acidity levels, and caffeine content were reduced. However, the total phenol and antioxidant activity increased approximately by 7.50% and 1.06% respectively as the increase of fermentation time and the addition of *L. mesenteroides* in roasted coffee beans. The best result was obtained at a 12-hour fermentation time due to the highest total phenols, antioxidant activity, and organoleptic score.

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REFERENCES

Aguilar-Zarate P, Cruz-Hernandez MA, Montañez JC, Belmares-Cerda RE, Aguilar CN. 2014. Enhancement of tannase production by *Lactobacillus plantarum* CIR1: Validation in gas-lift bioreactor. Bioprocess Biosyst Eng 37 (11): 2305-2316. DOI: 10.1007/s00449-014-1208-3.

Alkaltham MS, Özcan MM, Uslu N, Salamatullah AM, Hayat K. 2020. Effect of microwave and oven roasting methods on total phenol, antioxidant activity, phenolic compounds, and fatty acid compositions of coffee beans. J Food Process Preserv 44 (11): e14874. DOI: 10.1111/jfpp.14874.

Choi S, Jung S, Ko KS. 2018. Effects of coffee extracts with different roasting degrees on antioxidant and anti-inflammatory systems in mice. Nutrients 10 (3): 363. DOI: 10.3390/nu10030363.

De Bruyn F, Zhang SJ, Pothakos V, Torres J, Lambot C, Moroni AV, Callanan M, Sybesma W, Weckx S, De Vuyst L. 2016. Exploring the impacts of postharvest processing on the microbiota and metabolite profiles during green bean production. Appl Environ Microbiol 83 (1): e02398-16. DOI: 10.1128/aem.02398-16.

de Jesus Cassimiro DM, Batista NN, Fonseca HC, Naves JAO, Coelho JM, Bernardes PC, Dias DR, Schwan RF. 2023. Wet fermentation of *Coffea canephora* by lactic acid bacteria and yeasts using the Self-Induced Anaerobic Fermentation (SIAF) method enhances the coffee quality. Food Microbiol 110: 104161. DOI: 10.1016/j.fm.2022.104161.

Diviš P, Pořízka J, Křikala J. 2019. The effect of coffee beans roasting on its chemical composition. Potravinarstvo Slovak J Food Sci 13 (1): 344-350. DOI: 10.5219/1062.

Elhalis H, Cox J, Frank D, Zhao J. 2020. The crucial role of yeasts in the wet fermentation of coffee beans and quality. Intl J Food Microbiol 333: 108796. DOI: 10.1016/j.ijfoodmicro.2020.108796.

Fessard A, Kapoor A, Patche J, Assemat S, Hoarau M, Bourdon E, Bahorun T, Remize F. 2017. Lactic fermentation as an efficient tool to enhance the antioxidant activity of tropical fruit juice and teas. Microorganism 5 (2): 23. DOI: 10.3390/microorganisms5020023.

Galanakis CM. 2017. Handbook of Coffee Processing By-Products. Elsevier, London.

George D, Mallery P. 2018. IBM SPSS Statistics 25 Step by Step: A Simple Guide and Reference, 15th eds. Routledge, New York. DOI: 10.4324/9781351033909.

Gokulakrishnan S, Chandraraj K, Gummadi SN. 2005. Microbial and enzymatic method for the removal of caffeine. Enzyme Microb Technol 37 (2): 225-232. DOI: 10.1016/j.enzmictec.2005.03.004.

Hanif M, Heru, Utami H. 2019. Variation of time and mass of spent coffee grounds on leaching oil from instant coffee residue. Inovasi Pembangunan: Jurnal Kelitbangan 7 (1): 49-61. DOI: 10.35450/jip.v7i1.119. [Indonesian]

Hasanah M, Maharani B, Munarsih E. 2017. Antioxidant power of robusta coffee leaf extract and fraction (*Coffea robusta*) against DPPH (2,2-diphenyl-1-picrylhydrazyl) reagent. Indones J Pharm Sci Technol 4 (2): 42-49. DOI: 10.15416/ijpst.v4i2.10456. [Indonesian]

Higdon JV, Frei B. 2006. Coffee and health: A review of recent human research. Crit Rev Food Sci Nutr 46 (2): 101-123. DOI: 10.1080/10408390500400009.

Ibrahim I, Ayariga JA, Xu J, Boakai RK, Ajayi OS, Owusu-Kwarteng J. 2023. A comparative study of skimmed milk and cassava flour on the viability of freeze-dried lactic acid bacteria as starter cultures for yogurt fermentation. Foods 12: 1207. DOI: 10.3390/foods12061207.

Koduru L, Kim Y, Bang J, Lakshmanan M, Han NS, Lee D-Y. 2018. Genome-scale modeling and transcriptome analysis of *Leuconostoc mesenteroides* unravel the redox governed metabolic states in obligate heterofermentative lactic acid bacteria. Sci Rep 7 (1): 15721. DOI: 10.1038/s41598-017-16026-9.

Kostinek M, Specht I, Edward VA, Pinto C, Egounlety M, Sossa C, Mbugua S, Dortu C, Thonart P, Taljaard L, Mengu M, Franz CMAP, Holzapfel WH. 2007. Characterization and biochemical properties of predominant lactic acid bacteria from fermenting cassava for selection as starter cultures. Intl J Food Microbiol 114 (3): 342-351. DOI: 10.1016/j.ijfoodmicro.2006.09.029.

Kwak HS, Jeong Y, Kim M. 2018. Effect of yeast fermentation of green coffee beans on antioxidant activity and consumer acceptability. J Food Qual 2018 (1): 5967130. DOI: 10.1155/2018/5967130.

Latimer GW. 2023. Official Methods of Analysis of AOAC International. Oxford University Press, Oxford. DOI: 10.1093/9780197610145.001.0001.

Li X, Han Y, Wu X, Li L, Zhang R. 2023. Effects of inoculation with a binary mixture of *Lactobacillus plantarum* and *Leuconostoc citreum* on cell wall components of Chinese Dongbei suancai. Food Res Intl 173: 113458. DOI: 10.1016/j.foodres.2023.113458.

Liang N, Kitts DD. 2016. Role of chlorogenic acids in controlling oxidative and inflammatory stress conditions. Nutrients 8: 16. DOI: 10.3390/nu8010016.

Liao C, Tang X, Li M, Lu G, Huang X, Li L, Zhang M, Xie Y, Chen C, Li P. 2022. Effects of lactic acid bacteria, yeast, and their mixture on the chemical composition, fermentation quality, and bacterial community of cellulase-treated Pennisetum sinese silage. Front Microbiol 13: 1047072. DOI: 10.3389/fmicb.2022.1047072.

Liu Q, Zong C, Dong Z, Wu J, Zhu J, Li J, Zhang J, Shao T. 2020. Effects of cellulolytic lactic acid bacteria on the lignocellulose degradation, sugar profile and lactic acid fermentation of high-moisture alfalfa ensiled in low temperature seasons. Cellulose 27: 7955-7965. DOI: 10.1007/s10570-020-03350-z.

Liu SQ. 2016. Lactic acid bacteria: *Leuconostoc* spp. In: Reference Module in Food Science. National University of Singapore, Singapore.

Martins P, Jesus J, Santos S, Raposo LR, Roma-Rodrigues C, Baptista PV, Fernandes AR. 2015. Heterocyclic anticancer compounds: Recent advances and the paradigm shift towards the use of nanomedicine's toolbox. Molecules 20: 16852-16891. DOI: 10.3390/molecules200916852.

Mestanza M, Mori-Culqui PL, Chavez SG. 2023. Changes of polyphenols and antioxidants of arabica coffee varieties during roasting. Front Nutr 10: 1078701. DOI: 10.3389/fnut.2023.1078701.

Morales-Ramos V, Escamilla-Prado E, Ruiz-Carbajal RA, Pérez-Sato JA, Velázquez-Morales JA, Servín-Juárez R. 2020. On the soil-bean-cup relationships in *Coffea arabica* L. J Sci Food Agric 100 (15): 5434-5441. DOI: 10.1002/jsfa.10594.

Mousavi A, Pourakbar L, Moghaddam SS. 2022. Effects of malic acid and EDTA on oxidative stress and antioxidant enzymes of okra (*Abelmoschus escul*entus L.) exposed to cadmium stress. Ecotoxicol Environ Saf 248: 114320. DOI: 10.1016/j.ecoenv.2022.114320.

Naegele M. 2016. Determination of Caffeine in Coffee Products According to DIN 20481. Aglient Technologies Inc., Germany.

Nishiyama T, Hashiguchi Y, Sakata T, Sakaguchi T. 2003. Antioxidant activity of the fused heterocyclic compound 1,2,3,4-tetrahydroquinolines and related compounds-effect of ortho-substituents. Polym Degrad Stab 79 (2): 225-230. DOI: 10.1016/S0141-3910(02)00285-9.

Oestreich-Janzen S. 2010. Chemistry of coffee. In: Mander L (eds). Comprehensive Natural Products II Chemistry and Biology. Elsevier Ltd., Oxford.

Oliveira DM, Mota TR, Oliva B, Segato F, Marchiosi R, Ferrarese-Filho O, Faulds CB, Dos Santos WD. 2019. Feruloyl esterases: Biocatalysts to overcome biomass recalcitrance and for the production of bioactive compounds. Bioresour Technol 278: 408-423. DOI: 10.1016/j.biortech.2019.01.064.

Oliveira LS, Franca AS, Mendonça JCF, Barros-Júnior MC. 2006. Proximate composition and fatty acids profile of green and roasted defective coffee beans. LWT - Food Sci Technol 39 (3): 235-239. DOI: 10.1016/j.lwt.2005.01.011.

Park S-W, Park B-K, Ju Y-W, Benashvili M, Moon C-R, Lee S, Lee J, Son Y-J. 2025. Inoculation of starter cultures in dry processing enhanced the contents of bioactive compounds and sensory characteristics of Arabica coffee (*Coffea arabica* L.). Food Chem 475: 143226. DOI: 10.1016/j.foodchem.2025.143226.

Preedy VR. 2015. Coffee in Health and Disease Prevention. Elsevier Applied Science Publishers, London.

Purwoko T, Suranto, Setyaningsih R, Marliyana SD. 2022. Chlorogenic acid and caffeine content of fermented robusta bean. Biodiversitas 23 (2): 902-906. DOI: 10.13057/biodiv/d230231.

Purwoko T, Suranto, Setyaningsih R, Marliyana SD. 2023. Caffeine degradation by food microorganisms. Biodiversitas 24 (6): 3495-3502. DOI: 10.13057/biodiv/d240647.

Rao W, Fang Z, Chen Z, Wu J, Fang X. 2023. Antibacterial mechanism of metabolites of *Leuconostoc mesenteroides* against *Serratia liquefaciens*. LWT 187: 115335. DOI: 10.1016/j.lwt.2023.115335.

Reichembach LH, de Oliveira Petkowicz CL. 2020. Extraction and characterization of a pectin from coffee (*Coffea arabica* L.) pulp with gelling properties. Carbohydr Polym 245: 116473. DOI: 10.1016/j.carbpol.2020.116473.

Saanu AB. 2017. Pectinolytic activity of mutagenic strain of *Leuconostoc Mesenteroides* isolated from orange and banana fruit waste. J Appl Microbiol Biochem 2 (1): 1-6. DOI: 10.21767/2576-1412.100007.

Sari PDI, Swasti YR, Purwijantiningsih E. 2025. Antioxidant activity and quality of frozen yoghurt with *Moringa oleifera* Lamk. leaves extract. AgriTECH 45 (1): 31-40. DOI: 10.22146/agritech.83392.

Shen X, Wang Q, Wang H, Fang G, Li Y, Zhang J, Liu K. 2025. Microbial characteristics and functions in coffee fermentation: A review. Fermentation 11 (1): 5. DOI: 10.3390/fermentation11010005.

Sidoryk K, Jaromin A, Filipczak N, Cmoch P, Cybulski M. 2018. Synthesis and antioxidant activity of caffeic acid derivatives. Molecules 23 (9): 2199. DOI: 10.3390/molecules23092199.

Su H, Guo Y, Cheng H, Hu S, Zhang P, Yang Z. 2024. Probiotic and fermentation properties of *Leuconostoc mesenteroides* strain I1/53 from sugarcane juice by a multi-omics approach. LWT-Food Sci Technol 211: 116897. DOI: 10.1016/j.lwt.2024.116897.

Swasti YR, Leong LP, Purwijantiningsih E, Pranata FS. 2024. The effects of *Lactobacillus plantarum* addition to robusta coffee (*Coffea canephora*) during wet fermentation. Biodiversitas 25 (9): 3132-3140. DOI: 10.13057/biodiv/d250935.

Szlapinski SK, Charrette A, Guthrie N, Hilmas CJ. 2023. Paraxanthine safety and comparison to caffeine. Front Toxicol 5: 1117729. DOI: 10.3389/ftox 2023.1117729.

Tai E-S, Hsieh P-C, Sheu S-C. 2014. Effect of polygalacturonase and feruloyl esterase from *Aspergillus tubingensis* on demucilage and quality of coffee beans. Process Biochem 49 (8): 1274-1280. DOI: 10.1016/j.procbio.2014.05.001.

Tarigan IL, Ramadhan I, Pebridianto A, Latief M, Sutrisno S, Yusnaidar Y. 2023. Effect of fermentation on the quality of liberica coffee (*Coffea Liberica*) using *Lactobacillus Plantarum* and *Leuconostoc Mesenteroides* bacteria: Sensory analysis, volatile compound, antioxidant, and proximate analysis. SSRN. DOI: 10.2139/ssrn.4644595.

Utama CS, Zuprizal, Hanim C, Wihandoyo. 2018. Isolation and identification of lactic acid bacteria cellulolytic originated from fermented cabbage juice. Jurnal Aplikasi Teknologi Pangan 7 (1): 1-6. DOI: 10.17728/jatp.2155. [Indonesian]

Verster JC, Koenig J. 2018. Caffeine intake and its sources: A review of national representative studies. Crit Rev Food Sci Nutr 58 (8): 1250-1259. DOI: 10.1080/10408398.2016.1247252.

Wei F, Furihata K, Koda M, Hu F, Kato R, Miyakawa T, Tanokura M. 2012. ^{13}C NMR-based metabolomics for the classification of green coffee beans according to variety and origin. J Agric Food Chem 60 (40): 10118-10125. DOI: 10.1021/jf3033057.

Wibowo KF, Swasti YR, Pranata FS. 2023. Low fat and high protein meat analogue of cowpea (*Vigna unguiculata*) with stabilizer cocoa pod husk extract (*Theobroma cacao* L.). Food Res 7 (5): 1-11. DOI: 10.26656/fr.2017.7(5).789.

Yang F, Chen C, Ni D, Yang Y, Tian J, Li Y, Chen S, Ye X, Wang L. 2023. Effects of fermentation on bioactivity and the composition of polyphenols contained in polyphenol-rich foods: A review. Foods 12 (17): 3315. DOI: 10.3390/foods12173315.

Zhang H, Huang-Fu H-P, Wang X, Zhao S-S, Liu Y, Lv H, Qin G-Y, Tan Z. 2021. Antibacterial activity of lactic acid producing *Leuconostoc mesenteroides* qz1178 against pathogenic *Gallibacterium anatis*. Front Vet Sci 8: 630294. DOI: 10.3389/fvets.2021.630294.

Zhang SJ, De Bruyn F, Pothakos V, Contreras GF, Cai Z, Moccand C, Weckx S, De Vuyst L. 2019. Influence of various processing parameters on the microbial community dynamics, metabolomic profiles, and cup quality during wet coffee processing. Front Microbiol 10: 2621. DOI: 10.3389/fmicb.2019.02621.