

Changes in phenolic and flavonoid profiles and antioxidant activity of mango peel during *Lactobacillus plantarum* fermentation

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Abstract: Mango peel contains phenolic and flavonoid compounds with potential as natural antioxidants. However, most of these compounds remain bound as glycosides, which may reduce their bioactivity, particularly their antioxidant capacity. One approach to increasing the phenolic and flavonoid content of mango peel is through biotransformation using lactic acid bacteria (LAB), including *Lactobacillus plantarum*. This study aimed to analyze changes in phenolic and flavonoid contents and the metabolite profile of mango peel, as well as to evaluate its antioxidant activity following biotransformation by *L. plantarum*. We carried out the biotransformation and harvested samples at 0 and 60 h. We extracted the fermented samples using ultrasound-assisted extraction (UAE) with methanol and concentrated the extract by evaporation to obtain a crude extract. We then analyzed the crude extract for total phenolic content, total flavonoid content, metabolite profile by HPLC, and antioxidant activity using the FRAP and ABTS assays. The results showed that biotransformation increased total phenolic content by 4.8% and total flavonoid content by 18.84%, although the latter increase was not statistically significant. HPLC characterization supported these changes, revealing new peaks and changes in peak intensity following biotransformation. The increase in total phenolic and flavonoid contents was accompanied by enhanced antioxidant activity, as indicated by the increased reduction of Fe^{3+} to Fe^{2+} in FRAP assay and the improved scavenging activity against $\text{ABTS}^{\bullet+}$ radicals, reflected by a decrease in the IC_{50} value.

Keywords: *antioxidant; biotransformation; Lactobacillus plantarum; mango peel; phenolics*

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INTRODUCTION

Biotransformation is a field of biotechnology that has recently attracted considerable attention as a more environmentally friendly alternative to chemical synthesis (Roy et al., 2022). Chemical synthesis generally requires the use of organic solvents as well as high temperatures and pressures (Kharissova et al., 2019). Biotransformation is defined as the modification of the chemical structure of compounds by microorganisms or enzymes (Shanu-wilson et al., 2020). One of the biotransformation processes is microbial fermentation, which is used to improve the quality of food and herbal materials. This fermentation process can extend shelf life, improve organoleptic properties such as taste and aroma, enhance nutritional content, and reduce antinutritional compounds (Roy et al., 2022). Therefore, biotransformation-based fermentation serves not only as a preservation method but also as an approach to increase the added value of natural materials.

Phenolic compounds are secondary metabolites produced in plants through the shikimate pathway and exhibit various bioactive properties, including antioxidant, anticancer, anti-inflammatory, and other biological activities (Martins et al., 2011). Flavonoids are one of the major groups of polyphenolic compounds commonly found in plants (Panche et al., 2017). Flavonoids are potent antioxidants that can neutralize free radicals and suppress oxidative stress. However, phenolic compounds and their derivatives can occur in glycoside-bound forms, which exhibit lower antioxidant activity than their aglycone forms (Dias et al., 2021). Therefore, enzymatic biotransformation can modify or release phenolic and flavonoid compounds, enhancing their biological activity. Gao *et al.*

(2024) reported that the fermentation of alfalfa leaves with *L. plantarum* and *Pediococcus pentosaceus* increased the total flavonoid content by 17,93% through enzymatic activity that released bound flavonoids (Gao et al., 2024).

Lactic acid bacteria (LAB), including *Lactobacillus plantarum*, are widely used in the biotransformation of phenolic and flavonoid compounds through enzymatic activity, altering their metabolite profiles and biological activities (Anshory et al., 2025). These bacteria produce several enzymes involved in biotransformation, including amylase, esterase, β -glucosidase, phenolic acid decarboxylase (PAD) (Lu et al., 2025). Chen, *et al.*, (2023) reported that the fermentation of strawberry juice using *L. plantarum* and *L. acidophilus* converted caffeic acid into vanillic acid and *p*-coumaric acid, increasing antioxidant activity in the ABTS assay, with inhibition rates of 55% and 53%, respectively. Huang *et al.*, (2024) reported that the biotransformation of lychee pulp using *L. plantarum* and *L. fermentum* produced two new compounds, 3,5,7-trihydroxychromone and catechol, from quercetin, while increasing the total phenolic content and antioxidant activity.

Mango fruit is a potential source of flavonoids and phenolic compounds. Mangoes are cultivated in more than 100 countries worldwide, with Indonesia ranking third in mango production, producing 3.3 million tons in 2023, according to Indonesia's Badan Pusat Statistik (BPS). The most widely cultivated mangoes in Indonesia include *Mangifera indica* (Arummanis, Golek, Gedong, Manalagi, and Cengkir) and *Mangifera foetida* (Kemang and Kweni) (Masturi et al., 2019). Mango fruit is rich in bioactive compounds including mangiferin, catechin, quercetin, kaempferol, rhamnetin, gallic acid, ellagic acid, propyl gallate, methyl gallate, and protocatechuic acid, which contribute to its antioxidant capacity. High mango production in Indonesia also generates of waste, particularly peels and seeds, with mango peel accounting for 15-20 % of the total fruit weight. Rather than being discarded, mango peels could be valorized as a source of bioactive compounds, particularly phenolics and flavonoids, including mangiferin, quercetin, rhamnetin, ellagic acid, kaempferol, and rutin (Kabir et al., 2017). Despite their potential, the effective use of mango peel waste remains an important area for further investigation, particularly its antioxidant activity.

Biotransformation of mango peel can increase the value of agricultural waste while reducing environmental pollution. Microbial fermentation can modify and release phenolic and flavonoid compounds from the mango peel matrix, increasing their content and antioxidant activity. Vilas-franquesa, *et al.*, (2024) reported that fermentation of mango peel using *L. plantarum* and *Bifidobacterium animalis* spp., supplemented with Viscozyme and Pectinex, increased antioxidant activity by more than 30% through the release of glycosylated phenolic compounds. Therefore, biotransformation of mango peel is important to explore its potential as a source of bioactive compounds. This study aimed to analyze changes in total phenolic and flavonoid content; metabolite profiles using HPLC and LC-MS; and antioxidant activity using the FRAP and ABTS assays following mango peel biotransformation.

METHOD

Materials

The materials used in this study included mango peel from local market, *Lactobacillus plantarum*, Man Rogosa Sharpe Broth (MRSB), Man Rogosa Sharpe Agar (MRSA), saline (0,9% NaCl), sterile aquades, methanol, McFarland Standard solution, gallic acid standard solution, 10% Folin-Ciocalteu reagent, sodium carbonate (Na_2CO_3) solution, quercetin standard solution, 2% aluminum chloride (AlCl_3) solution, 1 M sodium acetate (CH_3COONa), 1% potassium ferricyanide, 0.2 M phosphate buffer (pH 6.6), 10 trichloroacetic acid (TCA) solution, 1% ferric chloride (FeCl_3) solution, Trolox standard solution, 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS), and potassium persulfate ($\text{K}_2\text{S}_2\text{O}_8$).

The instruments used in this study is knife, grinder (Philips), beaker glass (Iwaki), analytical balance, oven (LabTech), test tube (Iwaki), inoculating loop, incubator (Gallenkamp), incubator shaker (B-One SIC 50L Shaking Incubator), centrifuge, laminar air flow cabinet, petri dish, ultrasonic bath (Taffware), vials, rotary evaporator (Buchi), spectrophotometer UV-Vis (Shimadzu), hotplate stirrer (IKA® C-MAG HS4), micropipette, High Performance Liquid Chromatography/HPLC (Shimadzu), and Liquid Chromatography-Mass Spectrometry / LC-MS (Shimadzu LCMS-9050).

Methods

Lactobacillus plantarum was reactivated in MRS broth and incubated at 37 °C for 24 h. The bacterial culture was then inoculated onto MRS agar and incubated at 37 °C for 24 h to obtain single

colonies. Subsequently, the bacterial starter was prepared by suspending the bacterial cell in physiological saline and adjusting the suspension to the McFarland standard to obtain a cell concentration of 10^8 CFU/mL. The bacterial growth curve was then determined according to the method described by Risna *et al.*, (2022) by cultivating the bacteria in 150 mL of MRS broth, followed by measuring the optical density at 600 nm every 2 h using a UV-Vis spectrophotometer.

We collected bacterial colonies grown on agar with an inoculating loop and suspended them in sterile distilled water to prepare a bacterial suspension. A 30 μ L aliquot of the suspension was then pipetted onto a glass slide and heat-fixed over a spirit lamp. The smear was stained with crystal violet for 60 s and then rinsed with sterile distilled water until the purple color disappeared. Next, iodine solution was applied to the smear and allowed to stand for 30 s, then rinsed with sterile distilled water. The smear was then decolorized with alcohol for 30 s, rinsed again with sterile distilled water, counterstained with safranin for 60 s and finally rinsed with sterile distilled water and air-dried. The stained cells were observed under a light microscope (Li *et al.*, 2020).

This procedure was modified from Razola-D *et al.* (2024). The mango peels were washed, cut into small pieces, and dried in an oven at 40 °C until completely dry. The dried peels were then ground into powder and stored in a dry place at room temperature (Razola-Díaz *et al.*, 2024).

One gram of mango peel powder was added to 100 mL of sterile distilled water preheated to 70 °C and left to stand for 10 min. The suspension was sterilized by autoclaving at 121 °C for 20 min and cooled to room temperature. Subsequently, 3 mL of bacterial culture was inoculated into the sterile suspension to initiate fermentation at 37 °C with shaking at 120 rpm. We harvested the fermentation broth on day 0 (0 h) and day 3 (72 h). This procedure was modified from the methods described by Huang *et al.*, (2024) and Razola-Díaz *et al.*, (2024).

The fermentation products were extracted according to the method described by Huang *et al.* (2024) using the ultrasound-assisted extraction (UAE) method. The extraction was performed three times for 10 min in each cycle using chilled methanol at a sample-to-solvent ratio of 1:3. The extracts were then centrifuged to obtain the supernatants, which were combined and concentrated using a rotary evaporator to obtain a crude extract.

The total phenolic content was determined using the Folin-Ciocalteu method with gallic acid as the standard, according to the method described by Baturante *et al.*, (2023). Dissolve 10 mg of gallic acid in 10 mL of methanol (p.a.) to obtain a stock solution at 1000 mg/L. The stock solution was then diluted to prepare standard solutions at 0, 200, 400, 600, 800, and 1000 mg/L. The sample was prepared by dissolving 10 mg of the extract in 10 mL of methanol (p.a.) to obtain a concentration of 1000 mg/L. Subsequently, 1.5 mL of each standard solution and the diluted sample were transferred into a vial, then 0.4 mL of 10% Folin-Ciocalteu reagent was added. The mixture was homogenized and incubated for 10 min. After incubation, 4 mL of Na₂CO₃ solution was added; the mixture was homogenized, then 1 mL of distilled water was added. The resulting mixture was incubated at room temperature for 2 h. All standards and samples were analyzed in triplicate. Finally, the absorbance of each solution was measured using a UV-Vis spectrophotometer at a wavelength of 744.8 nm.

The total flavonoid content was determined using the AlCl₃ colorimetric method with quercetin as the standard, according to the method described by Baturante *et al.* (2023). A total of 10 mg of quercetin was dissolved in 10 mL of methanol to obtain a stock solution at 1000 mg/L. The stock solution was then diluted to prepare standard solutions with concentrations of 0, 5, 10, 15, 20, and 25 mg/L. The sample was prepared by dissolving 10 mg of the extract in 10 mL of methanol. Subsequently, 1.5 mL of each standard solution and sample was transferred into a vial, followed by the addition of 0.1 mL of 10% AlCl₃, 0.1 mL of 1 M sodium acetate, and 1.8 mL of distilled water, with homogenization after each reagent was added. The mixture was then incubated for 30 min. All standards and samples were analyzed in triplicate. After incubation, the absorbance of the solution was measured using a UV-Vis spectrophotometer at a wavelength of 431 nm.

The biotransformation products were characterized by HPLC analysis to evaluate changes in the phenolic and flavonoid compounds of mango peel following biotransformation. The HPLC system used was a Shimadzu LC-20Ai equipped with an SPD-40 UV-Vis detector. Separation was performed using a Shim-pack VP-ODS column (250 $\times$ 4.6 mm). The flow rate was set at 0.5 mL/min with an injection volume of 10 μ L. The mobile phase consisted of methanol and water (50:50, v/v).

To characterize changes resulting from biotransformation of mango peel using *L. Plantarum*, LC-MS analysis was also performed. The LC-MS instrument used was a Shimadzu LCMS-9050 equipped with a YMC-Triart C18 column (100 $\times$ 2.1 mm, S-1.9 μ m) as the stationary phase. The mobile phase consisted of acetonitrile and water containing 0.1% formic acid. The flow rate was set at 0.400

mL/min with an injection volume of 10 µL. The eluent gradient program was as follows: 0-5 min, 10%-90%; 10-20 min, 50-50%; 20-25 min, 50%-50%; and 25-30 min, 10%-90%.

Antioxidant Activity Assay

Antioxidant activity was evaluated using two methods, namely the FRAP assay according to Maryam S. et al. (2023) and the ABTS assay according to Wołosiak R, et al., (2022). The antioxidant activity was determined using the FRAP assay with Trolox as the standard. The standard solution was prepared by dissolving 10 mg of Trolox in 10 mL of methanol to obtain a stock solution with a concentration of 1000 mg/L. The stock solution was then diluted to prepare standard solutions with concentrations of 0, 20, 40, 60, 80, and 100 mg/L. The sample solution was prepared by dissolving 10 mg of the extract in 10 mL of methanol to obtain a concentration of 1000 mg/L. Subsequently, 1 mL of each standard solution and sample was transferred into a test tube. Then, 1 mL of 0.2 M phosphate buffer (pH 6.6) and 1 mL of 1% potassium ferricyanide were added. The mixture was homogenized and incubated at room temperature for 40 min. After incubation, 1 mL of 10% TCA solution was added, and the mixture was centrifuged at 3000 rpm. 1 mL of the upper layer was collected and mixed with 1 mL of distilled water and 0.5 mL of 1% FeCl₃ solution. All measurements were performed in triplicate. Finally, the absorbance was measured using a UV-Vis spectrophotometer at a wavelength of 700 nm. Antioxidant activity was calculated based on the Trolox standard curve and expressed as mg TE/g sample (Maryam, et al., 2023).

The antioxidant activity was determined using the ABTS assay with Trolox as the standard. In this method, K₂S₂O₈ activated the ABTS•⁺ radical. A total of 0.036 g of ABTS powder was dissolved in 10 mL of distilled water. Potassium persulfate (0.0066 g) was dissolved in 10 mL of distilled water. The two solutions were mixed and incubated in the dark for 12-16 h. The Trolox standard solution was prepared by dissolving 10 mg of Trolox in 10 mL of distilled water to obtain a stock solution with a concentration of 1000 mg/L. The stock solution was then diluted to prepare standard solutions with concentrations of 0, 20, 40, 60, 80, and 100 mg/L. The extract sample was prepared in the same manner by dissolving the mango peel extract to obtain a stock solution with a concentration of 1000 mg/L, followed by dilution to concentrations of 0, 20, 40, 60, 80, and 100 mg/L. Subsequently, 1 mL each of the Trolox standard solution and sample solution was transferred into a small vial, followed by the addition of 3 mL of the ABTS stock solution that had been incubated for 16 h. The mixture was then incubated for 5 min. after incubation, the absorbance was measured using a UV-Vis spectrophotometer at a wavelength of 743 nm. All standards and samples were analyzed in triplicate. The absorbance values obtained were used to calculate the percentage of inhibition using the following equation (Wołosiak et al., 2022):

$$\% \text{ Inhibition} = \frac{\text{Absorbance control} - \text{Absorbance sample}}{\text{Absorbance control}} \times 100\%$$

The IC₅₀ value was determined from the relationship between the sample concentration and the percentage of inhibition.

FINDINGS AND DISCUSSION

Lactobacillus plantarum

The reactivated bacterial culture exhibited colony characteristics typical of *L. plantarum*, including circular colonies with a milky white appearance, entire margins, a convex elevation, and a smooth, creamy texture. Gram staining observed under a light microscope at 100× magnification revealed purple, rod-shaped bacterial cells, indicating that the isolate was Gram-positive. Gram positive bacteria possess a thick cell wall with a high peptidoglycan content, enabling them to retain the crystal violet stain during the Gram staining procedure. The characteristics of the reactivated bacterial culture were consistent with those reported by Qian, *et al.* (2018), who described *L. plantarum* as a lactic acid bacterium forming milky white, circular, smooth colonies with a slightly convex elevation and exhibiting a Gram-positive reaction. *L. plantarum* is Gram-positive lactic acid bacterium. that produce of lactic acid from carbohydrate substrates during fermentation. *L. plantarum* is a facultative anaerobic, rod-shaped lactic acid bacterium with a width of approximately 0.9-1.2 µm and a length of approximately 3-8 µm (Anshory et al., 2025). These characteristics are consistent with light microscopy observations, which showed that the bacterial cells were rod-shaped. This finding further confirms that the identified isolate was *L. plantarum*. The colony morphology and cell morphology of the bacterium are presented in **Figure 1**.

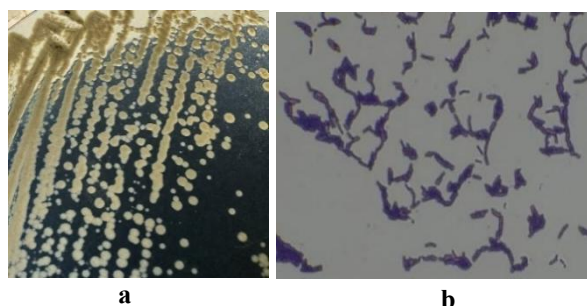


Figure 1. Colony morphology of *L. plantarum* (a) and cell morphology of *L. plantarum* (b)

A bacterial growth curve was constructed to determine the growth phase of *L. plantarum*, which served as the basis for selecting the fermentation time. The growth curve of *L. plantarum* is presented in **Figure 2**.

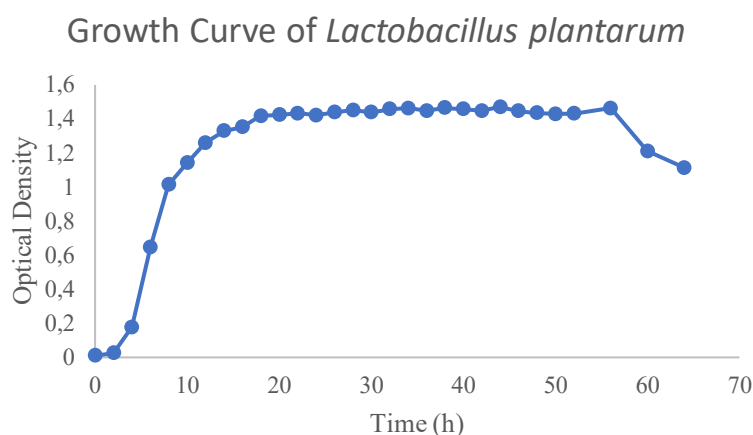


Figure 2. Growth curve of *Lactobacillus plantarum*

The lag phase occurred between 0 and 2 h, the exponential phase between 3 and 18 h, the stationary phase between 19 and 56 h, and the death phase between 57 and 60 h. Risna *et al.*, (2022) The initial or lag phase (0-2 h) is the stage at which bacteria adapt to their environment. During this phase, cell number does not increase significantly, although metabolic activity has already begun to support bacterial growth. During the exponential phase (3-18 h), bacteria are metabolically active, supporting optimal production of enzymes and metabolites involved in the biotransformation. The stationary phase (19-56 h) occurs when the growth and death rates become balanced, resulting in a stable cell population. Nutrient depletion and the accumulation of metabolic products, such as lactic acid, inhibit bacterial growth. Lactic acid production also alters environmental pH, affecting enzyme activity and enhancing secondary metabolite production or biotransformation under stress conditions. After 56 h, the culture entered the death phase, indicated by a sharp decline in the growth curve due to nutrient depletion and accumulation of toxic metabolites that no longer supported bacterial growth. Based on the growth curve, the fermentation was carried out for 60 h because the stationary phase represents the period during which enzymatic activity involved in biotransformation is considered optimal. Therefore, this fermentation time was selected to evaluate changes in phenolic and flavonoid contents, the metabolite profile, and the antioxidant activity of mango peel.

Extracts of Fermented Mango Peel

Fermentation was carried out under submerged conditions. During fermentation, *L. plantarum* produces lactic acid, which lowers the medium pH and may enhance enzyme activity and disrupt mango peel cell walls, facilitating enzyme access to phenolic and flavonoid compounds. Montijo-Prieto *et al.* reported that lactic acid bacteria enzymes increase phenolic and flavonoid contents during fermentation by disrupting plant cell walls, releasing bound phenolic compounds from avocado leaves. We extracted the biotransformation products using ultrasound-assisted extraction (UAE), focusing on extraction time, solvent consumption, and yield. Ultrasonic cavitation disrupts cell walls and facilitates compound diffusion from the plant matrix (Shen *et al.*, 2023). Its relatively low temperature also minimizes compound degradation. We selected methanol for ultrasonic extraction because it is a universal organic

solvent commonly used to extract polar compounds and is stable and resistant to oxidation (Chaves *et al.*, 2020). The UAE yielded 1.0245 g of crude extract from D-0 and 0.6214 g from D3. The lower extract yield after fermentation may be attributed to microbial and enzymatic activity that degrades complex components into simpler compounds the microorganisms use during fermentation. Esterase and β -glucosidase produced during fermentation can convert complex phenolic and flavonoid compounds into simpler, more soluble forms. Consequently, bioactive compounds may increase despite the lower total extract yield (Huynh *et al.*, 2014). The lower yield may also result from the degradation of some compounds during ultrasound-assisted extraction. This observation is supported by Zhang *et al.* (2015), who reported that gallic acid can degrade during ultrasonic extraction because increased cavitation intensity promotes free radicals in the extraction medium, which can react with compounds in the extraction medium.

Total Phenolic and Total Flavonoid Contents

Total Phenolic Content

The total phenolic content of mango peel powder before and after fermentation is presented in **Figure 3**.

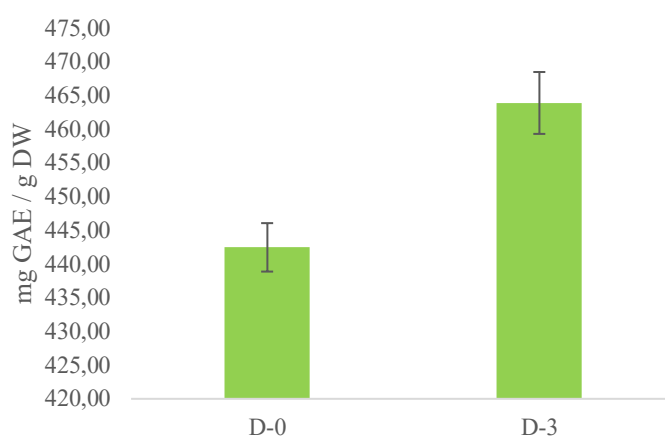


Figure 3. Bar chart of the total phenolic content (TPC) of mango peel powder before fermentation (D-0) and after fermentation (D-3). TPC increased significantly ($p < 0.05$). Values are presented as the mean $\pm$ standard deviation ($n = 3$).

Fermenting mango peel powder for 60 h increased total phenolic content by 4.8% (442.48 ± 3.6 to 463.90 ± 4.59 mg GAE/g DW). The increase in total phenolic content after fermentation likely reflects the activity of enzymes produced by *L. plantarum*, particularly esterase and β -glucosidase, which release bound phenolic compounds. Esterase catalyzes the cleavage of ester bonds between phenolic compounds and plant cell wall components, whereas β -glucosidase catalyzes the hydrolysis of glycosidic bonds associated with phenolic compounds, thereby increasing the amount of free phenolic compounds detected during the analysis (Lu *et al.*, 2025). A similar finding was reported by Razola-Díaz *et al.*, (2024), who observed that the fermentation of citrus peel using the lactic acid bacteria *L. plantarum* and *Lactobacillus brevis* increased the total phenolic content by 11% and 8%, respectively. In this study, we determined total phenolic content using the Folin-Ciocalteu method. The determination is based on the reaction between the hydroxyl groups of phenolic compounds and the Folin-Ciocalteu reagent under alkaline conditions, forming a dark blue molybdenum complex. The intensity of this dark blue color was measured using a UV-Vis spectrophotometer (Baturante *et al.*, 2023).

Total Flavonoid Content

The total flavonoid content of mango peel powder before and after fermentation is presented in **Figure 4**.

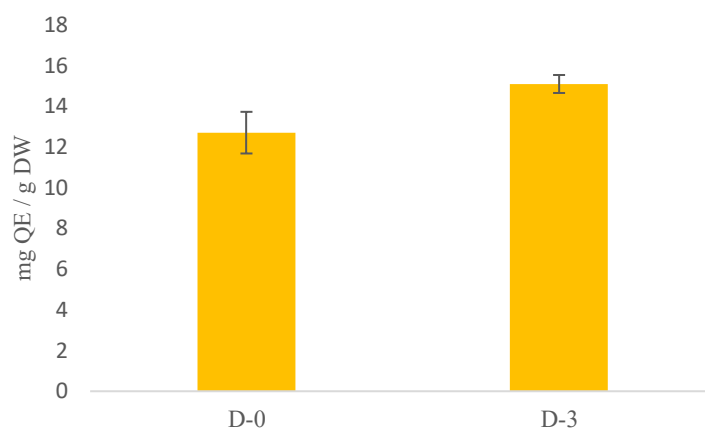


Figure 4. Bar chart of the total flavonoid content (TFC) of mango peel powder before fermentation (D-0) and after fermentation (D-3). The increase on total flavonoid content before and after fermentation was not statistically significant ($p > 0.05$). Values are presented as the mean $\pm$ standard deviation ($n=3$).

Following fermentation, the total flavonoid content increased by 18.83%, from 12.69 ± 1.02 mg QE/g DW to 15.08 ± 0.44 mg QE/g DW. However, this increase was not statistically significant ($p > 0.05$), indicating that *L. plantarum* biotransformation tended to increase the total flavonoid content without producing a significant change. Similarly, Ali S, *et al.* (2018), observed a non-significant increase in the total flavonoid content during the fermentation of longan fruit ($p > 0.05$). This suggests that fermentation may primarily modify flavonoid form rather than increasing their overall. The increase in total flavonoid content was likely associated with the activity of enzymes produced by *L. plantarum* during fermentation, particularly esterase and β -glucosidase, which facilitate the release of flavonoid compounds from their bound forms, thereby increasing the amount of flavonoids detected during the analysis (Lu, *et al.*, 2025). Gao *et al.*, (2024) Likewise, a 17.93% increase in the total flavonoid content during the fermentation of alfalfa leaves with *L. plantarum* and *Pediococcus pentosaceus* was reported, attributed to enzymatic modification of flavonoid structures.

The total flavonoid content was determined based on the formation of Al(III)-flavonoid complexes through interactions with keto and hydroxyl groups, producing changes in absorbance used for quantification. The formation of these complexes alters the optical properties of the compounds, leading to increased absorbance at a specific wavelength, which serves as the basis for the quantification of total flavonoid content (Nicolescu *et al.*, 2025). Although NaNO₂ and NaOH are commonly used in flavonoid assays, this study used sodium acetate. Sodium acetate was used to maintain a stable pH, thereby facilitating the formation of complexes between Al³⁺ and flavonoid compounds. Using sodium acetate emphasizes the formation of chelate complexes resulting from interactions between Al³⁺ and flavonoid functional groups. Consequently, this method is more specific for flavonoids containing free hydroxyl groups that interact directly with Al³⁺ (Anna & Pyrzynska, 2014).

Metabolite Profile of Biotransformed Mango Peel

We analyzed changes in the metabolite profile following fermentation using HPLC. The chromatograms obtained before and after fermentation are presented in **Figure 5**.

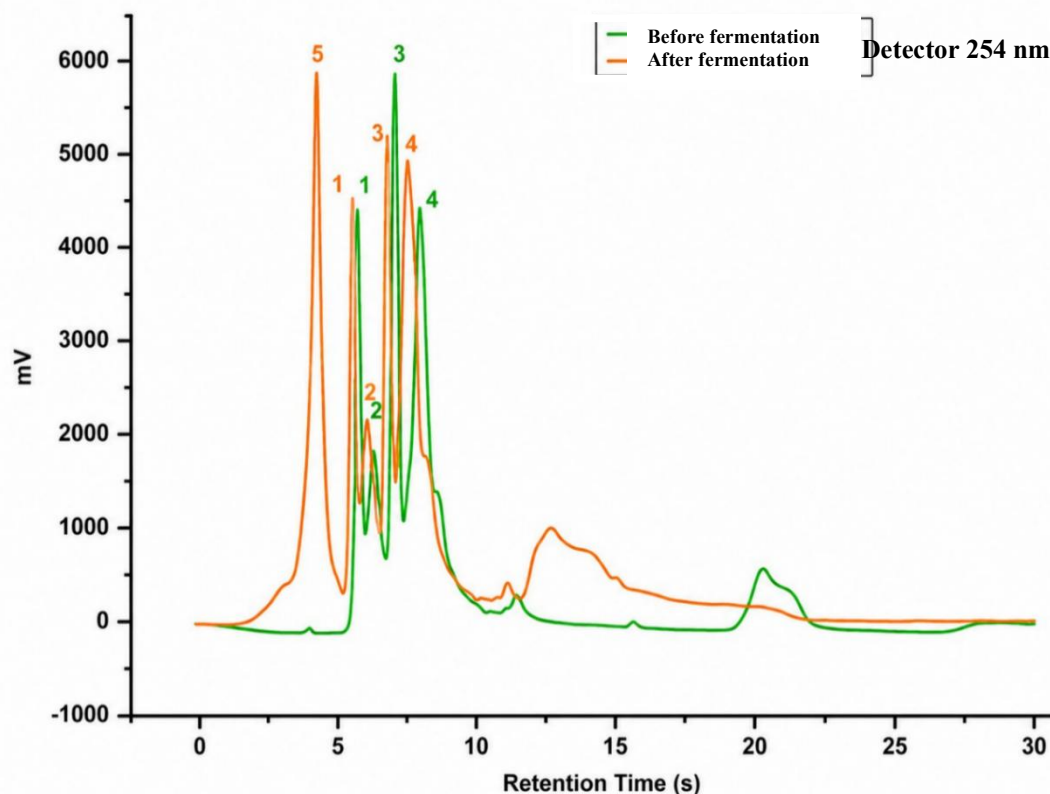


Figure 5. HPLC chromatogram mango peel before and after fermentation

The chromatograms before fermentation exhibited four peaks, whereas the chromatogram after fermentation showed five peaks. A peak at 5-9 min after fermentation was presumed to correspond to phenolic or flavonoid compounds commonly present in mango peel. The relatively early retention time may indicate higher polarity, as flavonoid glycosides generally elute earlier than their aglycone forms on a reversed-phase C18 column. Blunder, *et al.*, (2017) Flavonoid glycosides typically exhibit shorter retention times than flavonoid aglycones in HPLC. A new peak appeared at 3-4 min after fermentation, suggesting the formation of a new compound during the biotransformation. The earlier retention time suggests the compound was more polar than its precursor. Vacek, *et al.* (2012) Similarly, it was reported that biotransformation of flavonols increased their polarity through reactions such as glycosylation, methylation and sulfate conjugation, resulting in shorter HPLC retention times. Changes in the intensities of peaks 3 and 4 were also suggested to indicate changes in the abundance of the original compounds. Shin, *et al.* (2025), reported that the quercetin decreased during fermentation with *Bacillus licheniformis*, accompanied by the appearance of a new peak at a shorter retention time, suggesting the formation of a bioconversion product.

The peak at a retention time of 20 min shifted to approximately 13 min after fermentation, accompanied by an increase in peak intensity and peak broadening. This shift may reflect enzymatic modification of the compound composition and polarity during fermentation. However, HPLC retention times and peak intensities alone are insufficient to confirm compound identities. Therefore, LC-MS analysis was performed to obtain molecular mass and fragmentation information for more specific characterization of the metabolite changes. **Table 1** presents the LC-MS data of the compounds resulting from the biotransformation of mango peel.

Table 1. LC-MS data of compounds resulting from the biotransformation of mango peel with *L. Plantarum*

Retention Time (min)	[M-H] ⁻ <i>m/z</i>	Compound	Formula	Fragments	Response		Changes
					Before	After	
1.698	169.0139	Gallic acid	C ₇ H ₆ O ₅	79.018, 95.013, 124.016, 125.024, 168.005,	141.728	108.905	Decrease 23.2%
4.105	183.0296	Methyl gallate	C ₈ H ₈ O ₅	124.016, 123.008, 106.005, 173.023, 185.025,	ND	299.995	New
8.815	300.9988	Ellagic acid	C ₁₄ H ₆ O ₈	201.019, 229.014, 283.997	27.815	ND	Lost
1.543	331.067	Galloyl glucose	C ₁₃ H ₁₆ O ₁₀	124.016, 125.024, 169.014, 211.024, 148.053, 164.084,	61.228	34.330	Decrease 43.9%
12.511	293.1756	[6]-Gingerol	C ₁₇ H ₂₆ O ₄	177.092, 205.122, 220.147	52.266	38.225	Decrease 26.9%
9.028	469.053	unknown	-	169.014, 241.033	41.593	76.117	Increase 83%

ND : Not Detected. The compound was not detected either before or after fermentation

The LC-MS detected methyl gallate (*m/z* 183.026) after fermentation, with a response of 299.995, whereas the compound was not detected before fermentation. The appearance of methyl gallate may indicate the transformation of phenolic compounds during fermentation. This transformation is known to produce various enzymes involved in phenolic metabolism, including esterases and others. Methyl gallate is an ester gallic acid that can undergo further transformation during metabolism by *L. plantarum*s. Gallic acid was detected under both conditions but decrease by 23.2% from 141.728 to 108.905 possibly due to utilization or transformation by the bacteria during fermentation. Several studies have shown that *L. plantarum* can metabolize gallic acid into methyl gallate through enzymatic activity, including the transformation of gallic acid into derivatives such as pyrogallol (Felipe et al., 2017).

Changes in metabolites were also observed for ellagic acid, galloylglucose, [6]-gingerol, and one unidentified compound. Ellagic acid, which was detected before fermentation with a response of 27.815, was no longer detected after fermentation, while galloylglucose and [6]-gingerol decreased by 43.9% and 26.9%, respectively. In contrast, the unidentified compound increased by 83%, from 41.593 to 76.117. this pattern indicates that biotransformation does not only result in an increase in certain compounds but may also cause some compounds to decrease or disappear because they are used as substrates and undergo hydrolysis, decarboxylation, reduction, or changes in sugar or ester groups during microbial metabolism. The study conducted by Vilas-franquesa et al., (2024) reported that fermentation of mango peel with *L. plantarum* resulted in increased release of certain phenolic compounds, including gallic acid, and altered the overall metabolite profile. Overall, the LC-MS result support the HPLC findings that fermentation by

L. plantarum altered the metabolite profile of mango peel through the biotransformation process. These changes are also consistent with the result showing an increase in total phenolic content after fermentation, suggesting that the chromatographically observed changes in the metabolite profile may contribute to changes in the bioactivity of the extract.

Antioxidant Activity of Biotransformed Mango Peel

The antioxidant activity of mango peel extracts before and after fermentation was evaluated using the FRAP and ABTS assays to determine the effect of biotransformation on their antioxidant capacity.

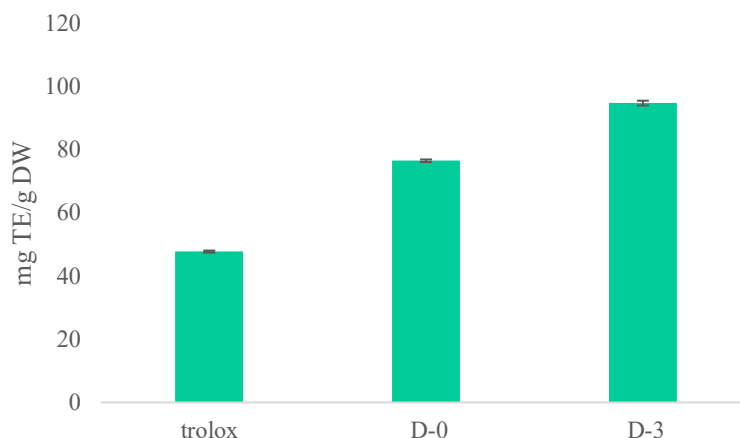


Figure 6. Bar chart of the antioxidant activity of the positive control (Trolox), before fermentation (D-0), and after fermentation (D-3) determined by the FRAP assay. The increase in antioxidant activity measured by the FRAP assay before and after fermentation was statistically significant ($p < 0.05$). values are presented as the mean $\pm$ standard deviation ($n = 3$)

Based on the results above, fermentation significantly increased the antioxidant activity measured by the FRAP assay ($p < 0.05$), from 76.5340 ± 0.41 mg TE/g DW before fermentation to 94.7778 ± 0.76 mg TE/g DW after fermentation. The results are expressed as mg Trolox Equivalent (TE) per gram of sample, indicating that the reducing capacity of 1 g of sample is equivalent to the antioxidant activity of Trolox used as the reference standard. The higher antioxidant activity observed after 3 days of fermentation indicates that the biotransformation of phenolic and flavonoid compounds enhanced the metal ion-reducing capacity compared with the initial condition (D-0). This increase in antioxidant activity was likely associated with the transformation or release of phenolic and flavonoid compounds through enzymatic activity, resulting in a greater number of free hydroxyl groups capable of donating electrons to reduce Fe^{3+} to Fe^{2+} . This finding is consistent with the observed increase in total phenolic and total flavonoid content following biotransformation (Ziekhrü et al., 2023). A similar result was reported by Montijo-Prieto *et al.* (2023), who found that the fermentation of avocado leaves using lactic acid bacteria, particularly *L. plantarum*, increased the antioxidant activity measured by the FRAP assay to 96.61 mg TE/g sample.

The antioxidant activity was further evaluated using the TEAC (Trolox Equivalent Antioxidant Capacity) assay, commonly referred to as the ABTS assay. In this method, Trolox was used as the reference standard, and the antioxidant activity was expressed as the IC_{50} value, as shown in Figure 7.

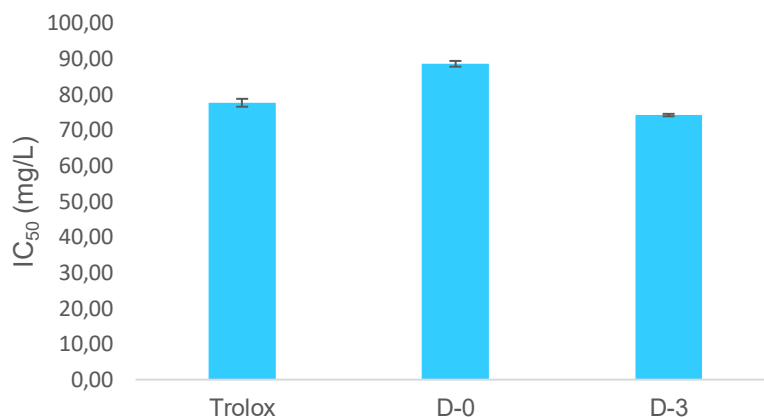


Figure 7. Bar chart of the antioxidant activity of the positive control (Trolox), before fermentation (D-0), and after fermentation (D-3) determined by the ABTS assay. The decreased in IC₅₀ values before and after fermentation was statistically significant ($p < 0.05$). Values are presented as the mean $\pm$ standard deviation ($n = 3$).

The antioxidant activity was evaluated using the ABTS assay to determine the ability of the compounds to scavenge free radicals through electron transfer or hydrogen atom transfer mechanisms. Antioxidant activity is indicated by a decrease in the intensity of the blue-green ABTS $\cdot^+$ radical to its colorless neutral form, which was subsequently measured using a UV-Vis spectrophotometer (Munteanu & Apetrei, 2021). The antioxidant activity was expressed as the IC₅₀ value defined as the concentration of the sample required to scavenge or neutralize 50% of the ABTS $\cdot^+$ radicals. Therefore, a lower IC₅₀ value decreased significantly ($p < 0.05$) from $88,6421 \pm 0,81$ mg/L for D-0 sample to $74,2445 \pm 0,34$ mg/L for D-3 sample. According to the standard classification of antioxidant activity, both samples exhibited strong antioxidant capacity because their IC₅₀ values were below 100 mg/L. the reduction in IC₅₀ indicates that biotransformation enhanced the antioxidant capacity of the mango peel extract to scavenge ABTS $\cdot^+$ radicals, resulting in a lower concentration being required to inhibit 50% of the radicals compared with the extract before fermentation (Wołosiak et al., 2022). This improvement in antioxidant activity was likely associated with the enzymatic activity of *L. plantarum* during the biotransformation process, which promoted the release of bound phenolic and flavonoid compounds and the formation of metabolites containing free hydroxyl groups. These hydroxyl groups act as electron donors making them effective in neutralizing ABTS $\cdot^+$ radicals. This finding is consistent with the study by Chen *et al.*, (2023), who reported that the biotransformation of strawberry juice using *L. plantarum* increased antioxidant activity in the ABTS assay, with an inhibition capacity 55% higher than that of the control.

Although the FRAP and ABS assays measure antioxidant activity through different mechanisms, both showed the same trend, namely an increase in antioxidant activity after fermentation. These results indicate that biotransformation with *L. plantarum* not only enhanced the metal ion-reducing capacity but also improves the free radical-scavenging activity of the mango peel extract, the increase in antioxidant activity was consistent with the observed increases in total phenolic and total flavonoid contents, as well as the changes in the metabolite profile revealed by HPLC analysis. Together, these findings support that fermentation was able to produce compounds with higher antioxidant activity.

CONCLUSION

Biotransformation using *L. plantarum* increased the phenolic and flavonoid content of mango peel. Total phenolic content increased by 4.8%, while total flavonoid content increased by 18.84%, although the difference was not statistically significant. Biotransformation also altered the mango peel metabolite profile, as shown by a new peak and changes in peak intensity in the HPLC chromatogram. LC-MS further confirmed these changes by detecting methyl gallate and changes in several other metabolites after fermentation. These changes in the metabolite profile were accompanied by a significant improvement in

antioxidant activity, reflected by an enhanced ability to reduce metal ions and scavenge ABTS^{•+} radicals.

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