Research Article

Co-culture fermentation-driven biotransformation of bioactive compounds in banana peel vinegar: Enhancing functional properties for halal food applications

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Article history:
Received: 25 May 2026
Revised: 5 June 2026
Accepted: 29 June 2026
Avalibale online: 30 June 2026

Keywords:
Banana peel vinegar
Co-culture fermentation
Lactobacillus plantarum
Halal food
Waste valorization

https://doi.org/xxxxx

Abstract

The valorization of agro-industrial by-products into functional food products represents an important strategy to support sustainable food systems and resource efficiency. Banana peel, a major fruit waste, is rich in bioactive compounds but remains underutilized due to limited bioavailability. This study aimed to investigate the biotransformation of bioactive compounds in banana peel vinegar produced through co-culture fermentation using *Saccharomyces cerevisiae* and *Lactobacillus plantarum*. Banana peel extract was subjected to alcoholic fermentation for 5 days followed by acetic fermentation for 8 days using *Acetobacter aceti*. The concentration of *L. plantarum* was varied (1%, 2%, and 4%) to evaluate its effect on physicochemical properties. The results showed that acetic acid content and pH were not significantly affected ($p > 0.05$), while antioxidant activity increased significantly at 2% concentration ($p < 0.05$). Total phenolic content decreased with increasing bacterial concentration, indicating the occurrence of microbial biotransformation during fermentation. Alcohol content increased during alcoholic fermentation and decreased to near-zero levels after acetic fermentation, confirming efficient ethanol conversion. The 2% treatment was identified as the optimal condition, providing the highest antioxidant activity and balanced physicochemical properties. These findings highlight the potential of banana peel as a sustainable raw material for functional vinegar production and support the development of halal-compliant fermentation processes through minimal residual alcohol and the use of food-grade microorganisms, contributing to environmentally sustainable and value-added food innovation.

1. INTRODUCTION

The increasing global demand for sustainable and functional food systems has intensified the exploration of agro-industrial by-products as alternative sources of bioactive compounds. Banana peel, representing approximately 30-40% of total fruit biomass, is one of the most abundant organic wastes generated worldwide. Despite being underutilized, banana peel is rich in phenolic compounds, flavonoids, tannins, and other phytochemicals with significant antioxidant potential, highlighting its promising role as a functional food ingredient (Hikal et al., 2022; Putri et al., 2023). However, the direct utilization of banana peel is limited due to its complex matrix and the presence of bound phenolic compounds with low bioavailability. Microbial fermentation has emerged as a powerful biotechnological approach to enhance the functional properties of plant-based substrates through biotransformation mechanisms. During fermentation, microorganisms enzymatically convert complex compounds into simpler and more bioavailable forms, thereby improving antioxidant capacity, nutritional quality, and biological activity (Adebo & Medina-Meza, 2020). In particular, lactic acid bacteria such as *Lactobacillus* *plantarum* are known to increase phenolic content and antioxidant activity through enzymatic hydrolysis and metabolic conversion pathways (Meng et al., 2025). Similarly, yeasts such as *Saccharomyces cerevisiae* play a crucial role in alcoholic fermentation, contributing to the production of intermediate metabolites that serve as precursors for further biotransformation processes.

Vinegar production provides a strategic platform for the valorization of fruit-based substrates into functional products with enhanced shelf life and bioactivity. Fruit vinegar is characterized by a complex matrix of organic acids, phenolic compounds, vitamins, and fermentation-derived metabolites that collectively contribute to its health-promoting properties (Liu et al., 2019). Although previous studies have explored banana-based vinegar production, most investigations focus on fruit pulp rather than peel waste, and primarily employ single-culture fermentation systems. Such approaches may limit microbial interactions and reduce the diversity of metabolites generated during fermentation. Co-culture fermentation, involving synergistic interactions between yeast and lactic acid bacteria, represents a more advanced strategy to enhance metabolic complexity and functional attributes. The interaction between *S. cerevisiae* and *L. plantarum* has been reported to improve physicochemical properties, modulate metabolite profiles, and enhance sensory characteristics in fermented products, including fruit vinegar systems (Laily et al., 2019; Pratiwi et al., 2025). These microbial consortia enable sequential and complementary metabolic pathways, leading to more extensive biotransformation of bioactive compounds compared to monoculture fermentation.

Despite these advancements, studies specifically addressing the biotransformation of bioactive compounds in banana peel vinegar using co-culture systems remain scarce. In particular, there is limited understanding of how microbial interactions influence phenolic transformation pathways and contribute to the enhancement of functional properties in the final product. This gap underscores the need for systematic investigation integrating fermentation technology with bioactive compound profiling. From a sustainability perspective, the valorization of banana peel into functional vinegar aligns with circular bioeconomy principles by reducing organic waste and promoting resource efficiency. Furthermore, fermentation-based processing offers a natural and clean-label approach that is compatible with evolving dietary frameworks emphasizing safety, quality, and ethical considerations in food production. Therefore, this study aims to investigate the biotransformation of bioactive compounds in banana peel vinegar produced through co-culture fermentation using *S. cerevisiae* and *L. plantarum*. The study further evaluates the changes in bioactive profiles and their implications for enhancing the functional properties of vinegar, providing a scientific basis for the development of sustainable and value-added functional food products.

2. MATERIALS AND METHODS

2.1 Materials and Microbial Culture

Ripe banana peels (*Musa paradisiaca*, Kepok variety) were collected from local markets in Surabaya, Indonesia. The peels were selected based on uniform ripeness (yellow stage), washed thoroughly, and processed immediately. Analytical-grade reagents were used for all chemical analyses. The microbial cultures used in this study included *Saccharomyces cerevisiae* (yeast) and *Lactobacillus plantarum* (lactic acid bacteria) for co-culture fermentation, as well as *Acetobacter aceti* for acetic acid fermentation. *S. cerevisiae* and *A. aceti* were cultivated in Yeast Extract Peptone Dextrose (YPD) broth, while *L. plantarum* was grown in De Man, Rogosa, and Sharpe (MRS) broth under controlled laboratory conditions prior to inoculation.

2.2 Preparation of Banana Peel Substrate

Banana peels were cut into small pieces and blended with distilled water at a ratio of 1:1 (w/v). The homogenate was filtered using muslin cloth and filter paper to obtain banana peel extract. The filtrate was pasteurized at 65 °C for 30 min to reduce microbial contamination. The total soluble solids of the extract were adjusted to 15 °Brix using glucose to ensure sufficient fermentable sugar for microbial metabolism.

2.3 Co-Culture Fermentation and Vinegar Production

Alcoholic fermentation was initiated by inoculating banana peel extract with *Saccharomyces cerevisiae* (2%, v/v) and *Lactobacillus plantarum* at varying concentrations (1%, 2%, and 4%, v/v), followed by incubation under anaerobic conditions at 37 °C for 5 days. The experiment was arranged in a randomized block design with three replicates per treatment. The resulting wine was centrifuged

(4722×g, 15 min), and the supernatant was subjected to acetic acid fermentation using *Acetobacter aceti* (4%, v/v) under aerobic conditions at 37 °C for 8 days. The final product was centrifuged (7387×g, 15 min) and pasteurized at 65 °C for 30 min to obtain banana peel vinegar (Y. Chen et al., 2017).

2.4 Physicochemical Analysis

Physicochemical properties were evaluated for banana peel extract, fermented wine, and the final vinegar. The pH was measured using a calibrated digital pH meter, while alcohol content was determined according to standard AOAC methods. Total sugar content was analyzed using standard spectrophotometric methods (Pratiwi et al., 2025).

2.5 Determination of Total Titratable Acidity

Total titratable acidity, expressed as acetic acid, was determined by titration using standardized NaOH solution with phenolphthalein as an indicator. The results were calculated and expressed as percentage of acetic acid (Pratiwi et al., 2025).

2.6 Determination of Antioxidant Activity and Total Phenolic Content

Antioxidant activity was determined using the DPPH radical scavenging assay. Briefly, 1 mL of sample was mixed with 1 mL of 0.1 mM DPPH solution in methanol and incubated in the dark at room temperature for 30 min. The absorbance was measured at 517 nm using a UV-Vis spectrophotometer. Radical scavenging activity was calculated as percentage inhibition relative to a control. Total phenolic content (TPC) was measured using the Folin-Ciocalteu method. An aliquot of sample (0.5 mL) was mixed with 2.5 mL of 10% Folin-Ciocalteu reagent and incubated for 5 min, followed by the addition of 2 mL of 7.5% sodium carbonate. The mixture was incubated at room temperature for 30 min, and absorbance was recorded at 765 nm. TPC was expressed as mg gallic acid equivalents (GAE) per liter based on a gallic acid calibration curve. All analyses were performed in triplicate (Zubaidah et al., 2024).

2.7 Statistical Analysis

All experiments were conducted in triplicate, and the results were expressed as mean ± standard deviation. Statistical differences among treatment groups were evaluated using one-way analysis of variance (ANOVA) at a significance level of $p < 0.05$. When significant differences were observed, Duncan's multiple range test (DMRT) was applied for post hoc comparisons. All statistical analyses were performed using appropriate statistical software.

3. RESULTS AND DISCUSSION

3.1 Effect of *Lactobacillus plantarum* Concentration on Physicochemical Properties of Banana Peel Vinegar

The physicochemical properties of banana peel vinegar under different concentrations of *L. plantarum* are presented in Table 1. The results showed that variations in inoculum concentration did not significantly affect acetic acid content and pH ($p > 0.05$), although slight variations were observed. The acetic acid content ranged from 0.34% to 0.35%, indicating a relatively stable acetic fermentation process across treatments. This stability suggests that the conversion of ethanol into acetic acid by acetic acid bacteria occurred efficiently and was not strongly influenced by the variation in *L. plantarum* concentration (Pratiwi et al., 2025). In contrast, antioxidant activity showed a significant increase at 2% concentration ($p < 0.05$), reaching 75.12%, compared to other treatments. This result indicates that moderate levels of *L. plantarum* may enhance the release or transformation of bioactive compounds during fermentation. The increase in antioxidant activity is commonly associated with microbial enzymatic activity that promotes the liberation of phenolic compounds or the formation of new antioxidant metabolites (Boonsupa et al., 2019).

Interestingly, total phenolic content showed a decreasing trend with increasing bacterial concentration.

This may be attributed to the degradation or transformation of phenolic compounds into simpler molecules during fermentation. Previous studies have reported that microbial metabolism can both increase and decrease phenolic content depending on the balance between synthesis and degradation pathways (Bakir et al., 2017). The pH values ranged from 3.02 to 3.15, indicating acidic conditions suitable for vinegar production. The slight decrease in pH with increasing bacterial concentration is consistent with the accumulation of organic acids during fermentation (Boondaeng et al., 2022).

Table 1. Physicochemical Properties of Banana Peel Vinegar

L. plantarum Inoculation	Acetic Acid (%)	Antioxidant Activity (%)	Total Phenolic (mg GAE/L)	pH
1%	0.34 ± 0.02[a]	68.31 ± 0.70[a]	88.00 ± 0.17[a]	3.15 ± 0.00[a]
2%	0.35 ± 0.02[a]	75.12 ± 1.47[b]	69.28 ± 0.54[b]	3.04 ± 0.02[b]
4%	0.34 ± 0.02[a]	70.05 ± 0.35[a]	52.33 ± 0.58[c]	3.02 ± 0.02[b]

Data are expressed as mean ± SD (n = 3). Different letters in the same column indicate significant differences ($p < 0.05$)

3.2 Changes in Alcohol Content during Alcoholic and Acetic Fermentation

The changes in alcohol content during fermentation are illustrated in Figure 1. Alcohol levels increased during alcoholic fermentation (day 5), reaching approximately 5.70-5.75%, and subsequently decreased to near-zero levels after acetic fermentation (day 14). This pattern clearly demonstrates the sequential fermentation process, where sugars are first converted into ethanol by *S.cerevisiae*, followed by the oxidation of ethanol into acetic acid by acetic acid bacteria (de Matos et al., 2017; Ranganathan, 2024). The reduction of alcohol content during the acetic fermentation stage indicates an efficient conversion process. The near depletion of ethanol suggests that the fermentation system was well-controlled and that oxygen availability during the acetic stage supported the activity of acetic acid bacteria. This finding is consistent with previous studies showing that acetic acid fermentation significantly reduces residual alcohol content in vinegar products (G. L. Chen et al., 2023; Yildiz, 2023). These results are important not only for product quality but also for safety and regulatory considerations, particularly in relation to residual alcohol levels in fermented food products.

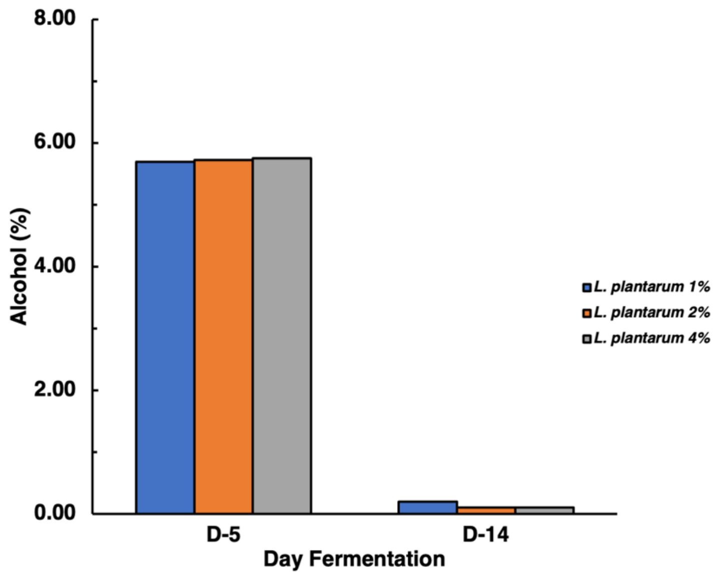

Figure 1. Alcohol content of banana peel vinegar at different fermentation stages (day 5 and day 14) with varying concentrations of *L.plantarum*. Values are expressed as mean ± SD (n = 3).

3.3 Determination of Optimal Treatment for Banana Peel Vinegar Production

Based on the overall results, the 2% *L. plantarum* treatment can be considered the optimal condition for banana peel vinegar production. This treatment exhibited the highest antioxidant activity while maintaining relatively stable acetic acid content and acceptable pH levels. Although the total phenolic content at 2% was lower than at 1%, the higher antioxidant activity suggests that other bioactive compounds or transformed phenolic derivatives may contribute to the antioxidant capacity. This indicates that antioxidant activity is not solely dependent on total phenolic content, but also on the structure and reactivity of the compounds present (Sharma et al., 2020; Tu et al., 2024). From a functional food perspective, the balance between antioxidant activity and physicochemical stability is essential. Therefore, the 2% treatment provides a favorable combination of bioactivity and product quality, making it suitable for further development as a functional vinegar product.

3.4 Implications for Halal-Compliant Fermentation Process

The fermentation process applied in this study also has important implications for halal-compliant food production. During alcoholic fermentation, ethanol is produced as an intermediate compound; however, it is subsequently converted into acetic acid during the acetic fermentation stage. The final alcohol content was found to be negligible, indicating that the ethanol was effectively metabolized. This transformation is critical in the context of halal food systems, where the presence of alcohol is a key concern. The near-complete conversion of ethanol into acetic acid supports the concept of a halal-compliant fermentation process, as the final product contains minimal residual alcohol (Ganesan et al., 2024; Hatta et al., 2023). Furthermore, the microorganisms used in this study, including *Saccharomyces cerevisiae* and *L. plantarum*, are widely recognized as food-grade and safe for consumption. The use of such microorganisms aligns with the principles of halal and clean-label food production, emphasizing safety, natural processing, and the absence of non-permissible substances (Leonés et al., 2019). These findings suggest that banana peel vinegar produced through co-culture fermentation has the potential to be developed as a halal-compliant functional food product.

4. CONCLUSION

The present study demonstrated that the concentration of *L. plantarum* in co-culture fermentation influenced the physicochemical properties of banana peel vinegar, particularly antioxidant activity and alcohol transformation. While acetic acid content and pH were not significantly affected, a significant increase in antioxidant activity was observed at 2% inoculum concentration, indicating enhanced bioactive compound transformation under moderate bacterial levels The fermentation process successfully followed a sequential pathway, where ethanol produced during alcoholic fermentation was effectively converted into acetic acid during acetic fermentation, resulting in negligible residual alcohol in the final product. This confirms the efficiency of the co-culture system in producing vinegar with desirable physicochemical characteristics. Among the tested treatments, the 2% *L. plantarum* concentration was identified as the optimal condition, providing the best balance between antioxidant activity and overall product quality. These findings highlight the potential of banana peel as a sustainable raw material for functional vinegar production. Furthermore, the near-complete conversion of ethanol into acetic acid supports the development of a halal-compliant fermentation process, as the final product contains minimal alcohol residue and utilizes food-grade microorganisms. Therefore, banana peel vinegar produced through co-culture fermentation can be considered a promising candidate for functional and halal-oriented food products.

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