Canrea Journal: Food Technology, Nutritions, and Culinary, 2025; 8 (2): 399–414 OPEN ACCESS

e-ISSN : 2621-9468

Type of the Paper (Article)

Improving the properties of coconut milk-based functional drinks with lactic acid bacteria fermentation and addition of herbal leaf

Muspirah Djalal[1,*], Dinda Amalia[1], Adila Oktavia[1], Mulyati M Tahir[1], Arfina Sukmawati Arifin[1], Serli Hatul Hidayat[1], Prakoso Adi[2], and Nurul Wakiah[3]

[1]Food Science and Technology, Faculty of Agricultural Technology, Hasanuddin University, 90245 Tamalanrea, Makassar, Indonesia
[2]International Doctoral Program in Agriculture, National Chung Hsing University, Taichung City, Taiwan
[3]Agricultural Product Technology, Faculty of Agriculture and Forestry, Sulawesi Barat University, 91413 Majene, Indonesia

Abstract

Coconut milk possesses potential as promising plant-based milk, alternative to animal milk. With the incorporation of bioactive-rich herbal leaves in combination with fermentation, the coconut milk presents an opportunity for better functional attributes, particularly antioxidant and antibacterial activities. This research investigates the effects of fermentation (lactic acid bacteria (culture)-mediated and spontaneous) and the addition of herbal leaves (cassava, bay, and guava) on the physicochemical and biofunctional properties of coconut milk drinks. Besides analysing antioxidant and antibacterial activities, other parameters analysed in this study include pH, total acidity, soluble solids (°Brix), total dissolved solids (ppm), and viscosity. This study finds that fermentation significantly reduced pH and the soluble solids (°Brix) while increasing total acidity, viscosity, and dissolved solids (ppm). It was also found that both fermentation and the addition of herbal leaves had significant effect on the biofunctional properties of the coconut milk drink in which they are found to enhance the antioxidant activity, with guava leaves exhibiting the highest enhancement. Particularly in guava-enriched formulations, the antibacterial properties were found to increase after fermentation demonstrating notable efficacy against *E. coli* (up to 7.03 mm) and *S. aureus* (up to 8.37 mm). This finding underscores the potential of fermentation in combination with herbal leaves to enhance the potential of plant-based milk.

Article History
Received June 23, 2025
Accepted December 9, 2025
Published December 31, 2025

Keywords
Antibacterial, Antioxidant, Coconut Milk, Functional Beverage, Herbal

1. Introduction

The interest in plant-based functional beverages has gained attention in recent years, which has led to research focused on providing safe and healthy plant-based food alternatives. There have been many studies reported of high interest, especially in plant-based milk, e.g., soy milk, almond milk, and coconut milk. Coconut milk, as one of the healthy food ingredients, serves as a potential base for functional beverages due to its rich nutritional profile, containing medium-chain triglycerides, essential vitamins, and minerals (1,2). Due to the high nutrition in coconut milk, it can be a good substrate for microbial fermentation. Fermentation can be applied to coconut milk as a strategy in the process of

* Correspondence : **Muspirah Djalal** muspirah_djalal@agri.unhas.ac.id

ISSN 2621-9468 online, DOI 10.20956/canrea.v8i2.1872

enhancing its functional properties, increasing shelf-life, and enriching its probiotic potential (3).

Several studies have reported the fermentation of coconut milk using various lactic acid bacteria (LAB) strains, such as *Lactobacillus plantarum*, *Lactobacillus acidophilus*, and *Streptococcus thermophilus*. Fermentation has been found to enhance their antioxidant and antibacterial activity (4–6). However, most of these studies still focused on the fermentation process alone to improve its functional properties, without further investigating the synergistic effects of other plants to see how the plant bioactive compounds can also alter the active properties in fermented coconut milk. To the best of our knowledge, currently, only a few studies have combined plant bioactive compounds with fermented coconut milk to enhance probiotic survival and functional properties and improve health benefits.

Thus, this study was conducted to investigate the combined effect of fermentation and the addition of herbal leaf extracts on the functional characteristics of coconut milk-based fermented functional beverages. Many of the herbal leaves around the world have been studied in their effect as antioxidant or antibacterial, include of them are cassava leaves (*Manihot esculenta*), guava leaves (*Psidium guajava*), and bay leaves (*Syzygium polyanthum*) that have been widely studied and shown to exhibit remarkable antioxidant and antibacterial activities due to their high content of flavonoids, tannins, and phenolic compounds. Cassava leaves are known to possess bioactive compounds such as alkaloids, phenolics, flavonoids, tannins, triterpenoids, quinones, and steroids (7). Guava leaves contain bioactive compounds such as quercetin, tannins, triterpenoids, eugenol, gallic acid, kaempferol, and malic acid, while bay leaves contain bioactive compounds such as eugenol and essential oils (8–11). However, its incorporation with coconut milk fermentation has not been further explored. It was expected that the addition of herbal leaf extracts containing bioactive compounds to fermented coconut milk beverages would increase antioxidant activity, improve antibacterial properties, and alter the physicochemical characteristics of fermented coconut milk beverages.

Especially with the incorporation of lactic acid bacteria culture fermentation, it is expected that due to their ability to survive in plant-based substrates, metabolize polyphenols, and increase bioactive compounds (12,13). They will also exhibit higher active properties of the fermented product due to the production of metabolites (14,15). The goal of this study is to find out how fermentation with lactic acid bacteria affects the antioxidant and antibacterial properties of coconut milk functional drinks, how adding herbal leaves affects the physical and chemical properties of fermented coconut milk, and whether the bioactive compounds in herbal leaves and probiotics can work together to improve the functional properties of coconut milk functional drinks. By achieving these objectives, this study is expected to provide new insights into formulating plant-based functional beverages, which can contribute to the development of probiotic-rich coconut milk beverages with enhanced bioactive properties.

2. Materials and Methods

The materials utilized in this study included plant-based substrates grated coconut from traditional market, bay leaves (*Syzygium polyanthum*), guava leaves (*Psidium guajava*), and cassava leaves (*Manihot esculenta*) all harvested in Makassar, Indonesia. The microbial strains employed comprised *Staphylococcus aureus ATCC 25923, Escherichia coli ATCC 25922*, and lactic acid bacteria isolated from fermented cabbage. Reagents and culture

media used in the study included, 75% ethanol, tetracycline (as a positive control for antibacterial test), de Man Rogosa and Sharpe Agar (MRSA, Merck), de Man Rogosa and Sharpe Broth (MRSB, HiMedia), 2,2-diphenyl-1-picrylhydrazyl (DPPH), 15% hydrogen peroxide (H_2O_2), phenolphthalein indicator, potassium carbonate (K_2CO_3), methanol (Merck), sodium hydroxide PA (Merck), Mueller-Hinton Agar (Oxoid), sodium chloride (Merck), Nutrient Agar (NA, Merck), and Nutrient Broth (NB, HiMedia).

2.1. Research Design

The study utilized a factorial completely randomized design (CRD) with two independent factors. The first factor was the type of herbal leaves, categorized into four levels: control (no herbal leaf addition), cassava leaves, bay leaves, and guava leaves. The second factor was the fermentation method, consisting of three levels: initial (unfermented), spontaneous fermentation, and controlled fermentation using a starter culture. Each treatment was performed in triplicate to ensure statistical validity and experimental reproducibility.

2.2. Research Procedure

2.2.1. Preparation of Coconut Milk

The preparation of coconut milk followed the method described by (16), with modifications. Grated coconut was mixed with distilled water at a 1:2 ratio. The mixture was then manually squeezed and filtered through a fine cloth to obtain coconut milk. Subsequently, 3% (w/v) sugar was added and homogenized. The coconut milk was then pasteurized at 75°C for 15 minutes and stored in a sealed container at 4°C until further use.

2.2.2. Preparation of Herbal Leaf Extract

The extraction of herbal leaves was performed following the method described by (17), with modifications. Fresh herbal leaves (cassava leaves, bay leaves, and guava leaves) were destemmed, cut small, and thoroughly washed with clean water to remove impurities. The leaves were then homogenized using a blender at a 1:1 ratio w/v. The homogenate was filtered and manually pressed using a filter cloth to obtain the extract. The resulting extract was pasteurized at 75°C for 15 minutes, rapidly cooled to room temperature, and stored in a hermetically sealed container at 4°C until further use.

2.2.3. Starter Preparation

The starter culture preparation was adapted from (18), with modifications. Purified lactic acid bacterial isolates were cultivated in MRS broth (MRSB) and incubated at 37°C for 48 hours. Bacterial growth was indicated by a change in medium color to cloudy. The culture was then centrifuged at 5000 rpm for 15 minutes, and the supernatant was discarded. The resulting cell pellet was washed with sterile physiological saline (0.9% NaCl) and subjected to a second centrifugation at 5000 rpm for 15 minutes. The final cell pellet was resuspended in sterile physiological saline (0.9% NaCl), and the optical density (OD) was measured at 620 nm using a spectrophotometer (Faithful 722 Vis Spectrophotometer) until an OD value of 1.2 was reached.

2.2.4. Fermentation

The fermentation process of functional beverages was adapted from (19), with modifications. Each herbal leaf extract (cassava leaves, bay leaves, and guava leaves) was incorporated into pasteurized coconut milk at a concentration of 30% (v/v). Subsequently, 5% (v/v) of the prepared starter culture was added and homogenized to ensure uniform mixing. The mixture was then incubated at 37°C for 48 hours. Following fermentation, the samples were process for analysis.

2.3. Analysis Parameters

2.3.1. pH

pH of the samples was measured using a digital pH meter (EZ-9908/686).

2.3.2. Total Acid

The total acid content was determined following the method outlined by (20). A 10 mL sample was precisely transferred into a 100 mL volumetric flask and diluted to the calibration mark with distilled water. Out of it, 25 mL sample was then taken and transferred into an Erlenmeyer flask. Subsequently, two drops of 1% phenolphthalein indicator were added. The sample was then titrated with 0.1 N NaOH until a pink color appeared. The total acid content was calculated using equation 1.

$$\text{Total Acids} = \frac{V1 \times N \times 90}{V2} \times Fp \times 100\% \quad (1)$$

Notes:

V1 = Volume NaOH (mL)
V2 = Volume sample (mL)
N = Normality NaOH (0.1)
Fp = Dilution Factor
90 = Mw of Lactic Acid

2.3.3. Total Soluble Solids (Total Sugar) (°Brix)

Total Soluble solid or total sugar (°Brix) was determined using a digital refractometer (Hanna HI-96814), following the method described by (21). The °Brix value was recorded from the digital display after measurement.

2.3.4. Total Dissolved Solids (ppm)

The total dissolved solids (TDS) content (ppm) was measured using a digital TDS meter (EZ-9908/686). The electrode probe was then activated and immersed in the sample until a stable reading was obtained and recorded.

2.3.5. Viscosity

Viscosity was determined using a digital viscometer (Brookfield RV-2M/2T Viscometer). The spindle (serial number 1) was securely attached, and the device was activated. The rotational speed was set to 30 rpm, and the spindle was immersed in a 100

mL sample until it reached the indentation limit mark. The spindle was then allowed to complete four full rotations before the viscosity value was recorded from the digital display.

2.3.6. Antioxidant Activity (% Inhibition)

Antioxidant activity was assessed using the DPPH radical scavenging assay, following the method described by Hanum, Yurliasni, and Seutia (2022) (22). A stock solution was prepared by dissolving 0.002 g of DPPH in 100 mL of methanol. 3.9 mL volume of 60 µM DPPH solution was pipetted into a test tube, followed by the addition of 100 µL of the sample. The mixture was homogenized and incubated in the dark for 30 minutes to prevent photodegradation. The absorbance of both the samples and blank was measured at 515 nm using a UV-Vis spectrophotometer (Faithful 722 Vis Spectrophotometer). The DPPH radical scavenging activity was subsequently calculated using equation 2.

$$\% \text{ Inhibition} = \frac{\text{A Blank} - \text{A Sample}}{\text{A Blank}} \times 100\% \quad (2)$$

2.3.7. Antibacterial Activity

Antibacterial activity was assessed using the disk diffusion method adapted from Elzuhria et al. (2023) (23), with modifications. A loopful of *Escherichia coli* and *Staphylococcus aureus* was inoculated into Nutrient Broth and incubated at 37°C for 48 hours to ensure optimal bacterial growth. The bacterial suspensions were then evenly spread onto Mueller-Hinton Agar (MHA) plates using a sterile cotton swab to achieve a uniform lawn culture. Sterile paper discs were impregnated with the test sample, sterile distilled water (negative control), and tetracycline (positive control) for 1 minute. The discs were subsequently placed onto the agar surface and incubated at 37°C for 24 hours. Antibacterial activity was quantified by measuring the diameter of the inhibition zone (clear zone) around each disc using a digital caliper. The samples showing larger zones indicate greater bacterial growth inhibition.

2.4. Data Analysis

The experimental data were subjected to statistical analysis using analysis of variance (ANOVA) to evaluate significant differences among the tested samples. When ANOVA indicated a statistically significant difference ($p < 0.05$), post hoc analysis was performed using Duncan's multiple range test to identify pairwise variations. All statistical analyses were conducted using Microsoft Excel 2021 and SPSS 25.0.

3. Results and Discussion

3.1. pH and Total Acid

During the fermentation process of lactic acid bacteria, sugar from the substrate is metabolized into organic acids, especially lactic acid, contributing to the increase of total acids and reduction of pH (24). In this study, pH and total acid were analysed to assess the change in acidification and determine the organic acid accumulation in coconut milk-based functional beverages before and after fermentation (Table 1).

Table 1. Changes in pH and total acid (%) of coconut milk-based functional beverages

Sample	pH			Total Acid (%)		
	Initial	Spontaneous	Inoculated	Initial	Spontaneous	Inoculated
Control	$5.71^{f} \pm 0.21$	$3.26^{c} \pm 0.08$	$2.29^{a} \pm 0.03$	$0.25^{a} \pm 0.04$	$0.58^{d} \pm 0.04$	$0.84^{f} \pm 0.02$
Cassava leaves	$5.13^{e} \pm 0.07$	$3.39^{c} \pm 0.21$	$2.21^{a} \pm 0.05$	$0.38^{b} \pm 0.02$	$0.62^{de} \pm 0.02$	$0.93^{g} \pm 0.04$
Bay Leaves	$5.12^{e} \pm 0.08$	$3.27^{c} \pm 0.01$	$2.34^{a} \pm 0.12$	$0.41^{b} \pm 0.02$	$0.61^{de} \pm 0.00$	$0.83^{f} \pm 0.05$
Guava Leaves	$5.53^{f} \pm 0.17$	$3.82^{d} \pm 0.18$	$2.88^{b} \pm 0.10$	$0.31^{a} \pm 0.03$	$0.53^{c} \pm 0.07$	$0.66^{e} \pm 0.09$

Notes: Values shown in the table are mean values (n=3). Values followed by different letters indicate significant differences at the 5% level ($p<0.05$).

Fermentation of coconut milk-based functional beverages, whether spontaneous or culture-driven, resulted in significant shifts in pH and total acid content, which are critical indicators of microbial metabolic activity. The initial or non-fermented sample shows the pH ranged from 5.12 to 5.71, differing with the difference of herbal leaves added, with the control sample exhibiting the highest value. Fermentation, both spontaneous and inoculated, led to a substantial pH reduction, with the highest acidification observed in the culture-fermented cassava leaf (2.21) and bay leaf (2.34) drinks, suggesting that these extracts may promote microbial acid production.

These findings emphasize the potential of particular herbal leaf extracts as fermentation enhancers, offering new paths for producing functional beverages with increased potential. Further characterization of microbial interactions and metabolite synthesis may provide deeper mechanistic insights into their role in functional beverage development.

The fermentation of coconut milk has been reported by several studies. Previous study by Mauro and Garcia., (2019) (25), reported a similar result in which coconut milk fermented with culture *Lactobacillus reuteri DSM 17938* showed a significant increase in acid production. The result found in this study indicates that controlled strains not only dominate the fermentation environment but also drive the metabolism towards acidification more effectively. Vitheejongjaeron, Phettakhu, Arsayot, and Taweechotipatr (2024) (26) investigated the application of *Lacticaseibacillus paracasei* in coconut milk fermentation and also reported a significant pH reduction. Deliberation inoculation not only accelerated the fermentation process but also led to a decrease in the pH level, contributing to the improvement of the flavor and preservation quality of the beverage.

3.3. Total Soluble Solid (Total Sugar) (°Brix) and Total Dissolved Solids (TDS) (ppm)

Total soluble solid or total sugar, measured in degrees Brix (°Brix) represents the concentration of soluble saccharides in a sample, which includes both reducing and non-reducing sugars. The °Brix value primarily represents the primary sugar constituents of the sample, which typically decrease during fermentation due to microbial metabolism. This measure is widely used in food and beverage analysis as an indicator of sugar content and overall product sweetness (27).

Total dissolved solids (TDS), measured in parts per million (ppm), indicate the total concentration of organic and inorganic solutes present in a liquid matrix (28). TDS analysis

was used in this study to monitor compositional changes in organic acids and other dissolved components as a result of fermentation. Unlike °Brix values, which mostly represent sugar concentration, TDS measurements provide a more comprehensive assessment of dissolved components, particularly organic acids, which tend to rise as fermentation occurs, resulting in higher TDS values.

The findings of total sugar (°Brix) and total dissolved solids (ppm) in coconut milk-based functional beverages are presented in Table 2.

Table 2. Changes in total sugar (°Brix) and total dissolved solids (ppm) in coconut milk-based functional beverages

Sample	Total Sugar (°Brix)			Total Dissolved Solids (ppm)		
	Initial	Spontaneous	Inoculated	Initial	Spontaneous	Inoculated
Control	$5.4^{f} \pm 0.4$	$5.2^{f} \pm 0.3$	$5.0e^{f} \pm 0.5$	$2990^{b} \pm 13$	$2638^{a} \pm 335$	$3316c^{d} \pm 248$
Cassava leaves	$4.5d^{e} \pm 0.1$	$3.6a^{b} \pm 0.7$	$3.2^{a} \pm 0.4$	$2938^{b} \pm 21$	$3271^{cd} \pm 89$	$3580^{d} \pm 214$
Bay Leaves	$3.9b^{c} \pm 0.2$	$3.3^{ab} \pm 0.2$	$3.3^{ab} \pm 0.1$	$3596^{d} \pm 30$	$4172^{e} \pm 178$	$4825^{f} \pm 36$
Guava Leaves	$4.2^{cd} \pm 0.1$	$3.8^{abc} \pm 0.2$	$3.3^{ab} \pm 0.2$	$2597^{a} \pm 255$	$3342^{cd} \pm 169$	$3158^{bc} \pm 104$

Notes: Values shown in the table are mean values (n=3). Values followed by different letters indicate significant differences at the 5% level (p<0.05).

Fermentation of coconut milk-based functional beverages, both spontaneously and with culture, resulted in significant changes in total sugars (°Brix) and total dissolved solids (TDS, ppm), indicating a complex interaction between microbial metabolism, phytochemical content, and fermentation conditions. ANOVA showed statistically significant differences ($p<0.05$), underscoring the impact of controlled fermentation and herbal supplementation on fermentation efficiency. Initially, total sugars ranged from 3.9 to 5.4 °Brix, with the control sample showing the highest value. Fermentation resulted in a signification reduction in total sugars, with the lowest total sugars in the culture-fermented cassava leaf drink (3.2) indicating that this extract can experience lower total sugars by microbial metabolism. The reduction in total sugar (°Brix) can be caused by the activity of lactic acid bacteria during the fermentation process. Lactic acid bacteria degrades simple sugars into organic acids (29). Lactic acid continues to increase during the fermentation process due to the activity of lactic acid bacteria, resulting in a decrease in total sugar (°Brix) during fermentation. This supported by the increase in the total acids and reduced pH after fermentation (Table 1).

A gradual reduction in total sugar was noted in all treatments, indicating culture-mediated metabolism of carbohydrate. But the degree of sugar loss was not the same in all treatments. The control reduced to 5.0 °Brix (0.4 °Brix decrease) which represents background metabolic conversion of fermentable carbohydrates to organic acids. The most significant reduction was found in the sample supplemented with the cassava leaf from 4.5 to 3.2 °Brix, and sample treated with guava leaves from 4.2 to 3.3 °Brix, while with incorporated bay leaves the reduction was from 3.9 to 3.3 °Brix. The faster sugar reduction in the cassava and guava leaf samples also implies the possibility of activating bacterial enzymes potential caused by their phytochemical content which may have enhanced microbial enzymatic activity.

Previous research has shown that extracts high in polyphenols can speed up the breakdown of sugars and the process of fermentation by activating bacterial amylase and invertase (30). The presence of phenolic acids, flavonoids, and alkaloids in cassava and guava leaves were suspected to contribute to this enhanced sugar metabolism, while the slightly slower decrease in bay leaf samples suggests the presence of antimicrobial compounds such as eugenol and flavonoids, which are known to modulate microbial activity (31–33).

Total dissolved solids (TDS), which showed an increase in all samples due to metabolites accumulated as a result of fermentation due to the activity of lactic acid bacteria, showed varying results depending on the herbal extract used. Bay leaf fermentation showed the highest increase in TDS to 4825 ppm, likely due to its rich bioactive content, organic acids, and peptides, significantly higher than the control sample ($p<0.05$). Cassava and guava leaf extracts also had a significant increase in TDS to 3580 ppm and 3158 ppm, respectively, indicating active microbial metabolism. The control sample showed the lowest TDS increase of 2990 to 3316 ppm, reinforcing the role of herbal extracts in increasing metabolite production. The existence of a strong correlation between the increase in TDS and the decrease in total sugar may further support the hypothesis that increased microbial metabolism leads to greater accumulation of fermentation product. Fermentation is a biological process that breaks down complex organic matter into simpler, more soluble compounds, such as organic acids, sugars, and ions. This process converts large fragments (e.g., fiber, proteins, polysaccharides) into small, air-soluble molecules, thereby increasing the Total Dissolved Solids (TDS) value (34). Similar results have been reported in plant-based fermented beverages, where a polyphenol-rich matrix enhances microbial metabolic activity and the solubilization of metabolites (35). However, the unequal trends of increasing TDS and decreasing Total soluble solid require further investigation of other factors that may be influential.

3.5. Viscosity

Viscosity is an important rheological parameter that determines a fluid's resistance to deformation and flow, governed by internal friction and molecular interactions (36). Viscosity serves as an indicator of the structural integrity of the sample, with higher viscosity values signaling increased fluid thickness and reduced flowability. In this study, viscosity analysis was conducted to evaluate the rheological modifications in coconut milk-based functional beverages caused by the fermentation process. The results of the viscosity assessment are presented in Figure 1.

Figure 1 indicated that a significant increase in viscosity could be observed in all fermented samples. The general reduced viscosity of all samples after mixing with herbal leaf extract was due to the lower viscosity of herbal leaf extract that affect the viscosity of beverage after mixture. However, we can clearly see from all samples that all experienced increase in viscosity after fermentation and higher increase in cultured sample, suggesting that microbial activity alone contributed to the increase in viscosity value, most likely through the production of exopolysaccharides (EPS). As has been reported in previous that several microbial strains can synthesize glucans and fructans, such as dextran, levan, and kefiran, which contribute naturally as rheology modifiers in fermented beverages (37,38).

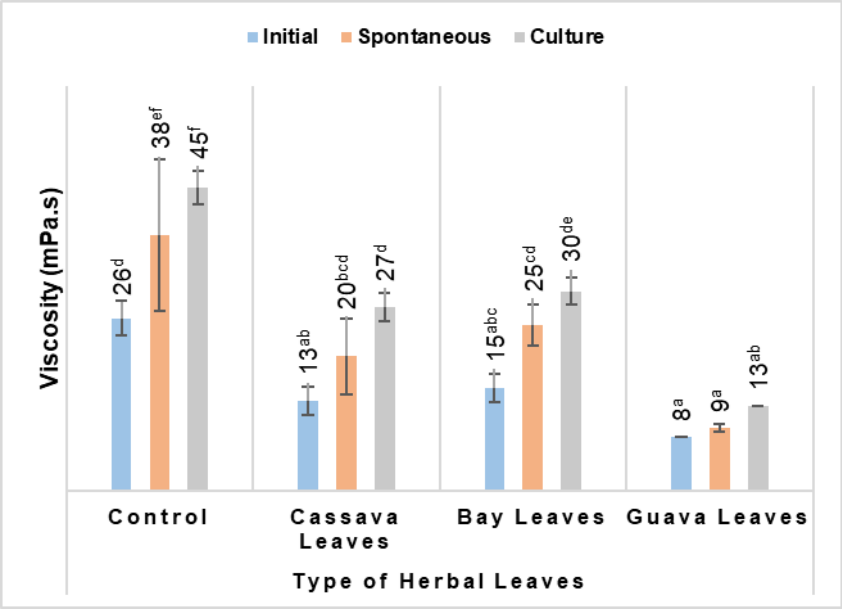

Figure 1. Viscosity Changes (mPa.s) in Coconut Milk-Based Functional Beverages; values shown in the graph are mean values (n=3). Values followed by different letters indicate significant differences at the 5% level ($p<0.05$).

3.6. Antioxidant Activity (% Inhibition)

In this study, antioxidant activity was evaluated to determine the biochemical changes in coconut milk-based functional beverages before and after fermentation. The results of the antioxidant activity assay are presented in Figure 2.

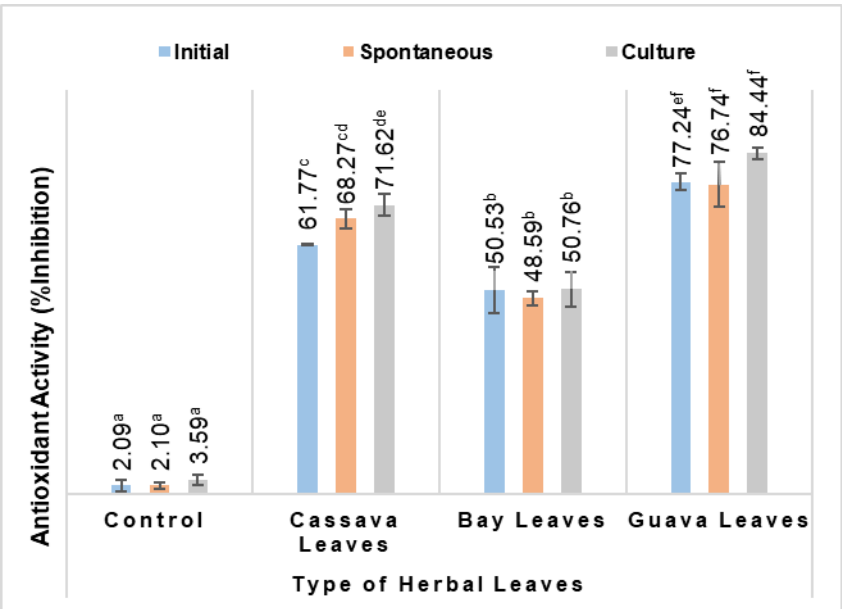

Figure 2. Enhancement of antioxidant activity (% inhibition) in coconut milk-based functional beverages; values shown in the graph are mean values (n=3). Values followed by different letters indicate significant differences at the 5% level ($p<0.05$).

The increase in antioxidant activity in fermented coconut milk-based functional beverages demonstrates the role of microbial metabolism in increasing the bioavailability of antioxidant compounds as a result of the fermentation process. As illustrated in Figure 2,

antioxidant activity with the incorporation of herbal leaves consistently resulted in higher antioxidant activity ($p < 0.05$) compared to control. This can be seen from the antioxidant activity of each sample: control from 2.09% to 3.59%, cassava leaves from 61.17% to 71.12%, bay leaves from 50.53% to 50.76%, and guava leaves from 77.24% to 84.44%. The control sample exhibited only a minor increase in antioxidant activity (2.09% to 3.59%) after fermentation, suggesting that microbial metabolism alone had a limited impact on antioxidant enhancement. This small increase may be attributed to the absence of herbal leaves that could contain phenolic compound that could promotes antioxidant properties. However, the significantly higher increase in antioxidant activity in the sample supplemented with herbal leaves indicates that phytochemical composition in herbal leaves could be is a major determinant in the increase of antioxidant potential (39–41).

Cassava leaf supplementation showed the greatest increase in antioxidant activity, which increased from 61.17% to 71.12%, indicating an increase in radical scavenging potential. This suggests that microbial hydrolysis facilitates the release and alteration of bound polyphenols, thereby increasing their potential antioxidant activity (42). The controlled fermentation process showed a higher antioxidant enhancement (71.12%) than spontaneous fermentation (68.72%), which reinforces the importance of targeted microbial selection in optimizing polyphenol conversion efficiency.

Bay leaf supplementation resulted in lowest antioxidant activity compared to the supplementation with other two herbal leaves in this study exhibiting from 50.53% to 50.76%, suggesting that its phenolic compound composition was less susceptible to microbial transformation.

On the other hand, guava leaf supplementation showed the highest initial antioxidant activity of 77.24% indicating an increase in radical scavenging potential, and after culture fermentation resulted in a slight increase of 84.44%. This relatively smaller increase indicates that guava leaves already contain high antioxidant compounds, such as flavonoids, ellagic acid, and vitamin C, thus requiring only minimal microbial transformation (43–45). The increase in antioxidant activity in fermented guava leaf sample cultures suggests that through bacterial metabolism, there may have been enzymatic release of bound phenolic compounds or alteration of precursor polyphenols into more bioactive derivatives. This is in line with previous studies showing that lactic acid bacteria (LAB) have glycosidases, phenolic acid decarboxylases, and esterases that can hydrolyse glycosylated phenolics into their aglycone form, thus increasing antioxidant potential (46).

The increased antioxidant activity in samples supplemented with herbal leaves can be attributed to a key biochemical mechanism. This mechanism is microbial enzymatic hydrolysis, which plays a crucial role in breaking down polyphenols and glycosylated polymers, thereby increasing their bioavailability. Furthermore, microbial β-glucosidases, tannases, and esterases catalyse the release of aglycones, which have greater free radical scavenging potential (30,47–49). The strong correlation between polyphenol content and antioxidant activity in cassava leaves supports the notion that microbial enzymes efficiently hydrolyse polyphenols. However, further research is necessary to analyse the correlation between phenolic compounds and antioxidant activity.

3.7. Antibacterial Activity

Herbal leaves are known for their properties as ethnomedicine that makes them used by people for treat certain medical problem. This is due to their rich in secondary metabolites with proven for example as antibacterial properties. In this study, antibacterial activity was assessed to determine the effect of herbal leaf extract and fermentation type on the antibacterial activity of coconut milk-based functional beverages before and after fermentation. The results of the antibacterial assay are presented in Figure 3.

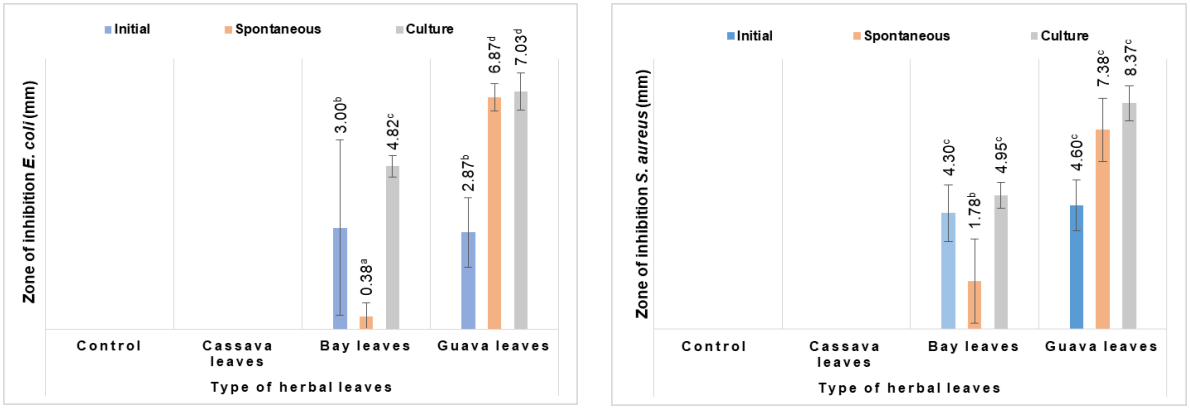

Figure 3. Antibacterial activity of coconut milk-based functional beverages fermented against *Escherichia coli* (a) and *Staphylococcus aureus* (b).

The antibacterial activity obtained was measured as zones of inhibition (mm) against *Escherichia coli* (Figure 3a) and *Staphylococcus aureus* (Figure 3b). The antibacterial activity results obtained showed a significant increase in zone of inhibition in all treatments ($p<0.05$) with controlled fermentation consistently producing the highest antibacterial effect.

Both control and the addition of cassava leaves shows no antibacterial activity while addition of other two herbal leaves show higher activity indicating the antibacterial activity strongly affected by the type of herbal leaves. Besides, a significant increase in antibacterial activity could be observed in all fermented samples ($p < 0.05$), confirming that fermentation enhanced the antibacterial efficacy beyond the initial activity of the herbal leaf extract alone. Fermented coconut milk-based beverages showed significant increases in both spontaneous fermentation and culture fermentation, with the highest antibacterial activity in cultured fermentation. Guava leaf supplementation showed the highest antibacterial activity against *E. coli* bacteria. This suggests that the microbial metabolic process aided in the release of flavonoids, tannins and volatile oil compounds, which are able to enhance the antimicrobial potential of the functional beverage. In comparison, bay leaf supplementation produced lower antibacterial activity, with the zone of inhibition increasing from 3.00 mm to 4.82 mm. Further analysis of detail compound of each product will be necessary to understand the mechanisms of the antibacterial efficacy.

Antibacterial activity against *S. aureus* bacteria, the result of guava leaf supplementation functional drink also showed the strongest antibacterial activity of 8.37 mm, followed by bay leaf of 4.95 mm. This was shown by the controlled fermentation treatment which consistently showed a significant increase in inhibition zone compared to spontaneous fermentation, which indicates that certain microbial strains can optimize the release of antimicrobial compounds. This strong antibacterial effect shown by coconut milk

formulated with guava leaves could possibly be due to the high content of flavonoids (quercetin, catechins), tannins, and terpenoids in guava leaves, which exhibit strong antimicrobial activity against both Gram-positive and Gram-negative bacteria (50).

The increased antibacterial activity across fermented samples can be attributed to multiple synergistic mechanisms. Microbial enzymatic activation of polyphenols plays an important role, fermentation can hydrolyse glycosylated flavonoids, converting them into aglycone forms with enhanced membrane-disrupting properties. Enzymes such as β-glucosidases, tannases, and esterases can aid the release of bioactive polyphenols, enhancing their antibacterial activity (30,51,52). In addition, fermentation-derived antimicrobial metabolites contribute significantly to antibacterial activity. Lactic acid bacteria (LAB) are capable of producing bacteriocins and organic acids (lactic acid), which can disrupt bacterial membranes and control fermentation processes that can optimize LAB metabolism, leading to the production of more bacteriocins and a decrease in pH value (53–55).

4. Conclusions

This study indicated that fermentation of coconut milk-based functional beverages with the incorporation of herbal leaves can improve the functional properties and alter the physicochemical properties of beverages produced. The results show a significant increase in both antioxidant and antibacterial properties, as well as an effect on physicochemical characteristics. The addition of herbal leaves to fermented beverages significantly enhances their functional value, with guava leaves showing the highest antioxidant and antibacterial activities among the other formulations tested in this study. However, we find that for future research analysis on detailed compound of the fermented product will be necessary to provide more idea on the mechanism behind the enhancement of the antioxidant and antibacterial activity.

Acknowledgments

The authors of this article extend gratitude to Prof. Andi Dirpan for facilitating laboratory equipment for use during the research.

Author Contributions

M.D. conceptualization, methodology, investigation, formal analysis, visualization, writing–original draft, writing–review and editing; D.A. writing–original draft, methodology, investigation, formal analysis, visualization; A.O. writing–original draft, writing–review and editing, data curation, visualization; M.M.T. validation, methodology; A.S.A. supervision, methodology, data curation; S.H.H. supervision, methodology, investigation, formal analysis, data curation; P.A. writing–review and editing, data curation; N.W. writing–review and editing, data curation.

Funding

The study was funded by LPPM Hasanuddin University. Hibah Penelitian Dosen Pemula Unhas (PDPU), Nomor: 00310/UN4.22/PT.01.03/2024.

Data Availability Statement

All data is presented in the article.

Conflicts of Interest

The authors declare no conflict of interest that could affect the results presented in the article.

References

1. Lu Y, Zhang Y, Wang S. From Palm to Plate: Unveiling the Potential of Coconut as a Plant-Based Food Alternative. J Agric Food Chem. 2024;72(27):15058–76.
2. Velangi M, Savla M. Role of Plant Based Milk Alternatives as a Functional Beverage: A Review. Int J Heal Sci Res. 2022;12(11):273–81.
3. Liu Y, Nawazish H, Farid MS, Qadoos KA, Habiba UE, Muzamil M, et al. Health-Promoting Effects of Lactobacillus Acidophilus and Its Technological Applications in Fermented Food Products and Beverages. Fermentation. 2024;10(8):380.
4. Qadi WSM, Mediani A, Benchoula K, Wong EH, Misnan NM, Sani NA. Characterization of Physicochemical, Biological, and Chemical Changes Associated with Coconut Milk Fermentation and Correlation Revealed by 1H NMR-Based Metabolomics. Foods. 2023;12(10):1–24.
5. Pachekrepapol U, Kokhuenkhan Y, Ongsawat J. Formulation of yogurt-like product from coconut milk and evaluation of physicochemical, rheological, and sensory properties. Int J Gastron Food Sci. 2021;25:100393.
6. Khubber S, Marti-Quijal FJ, Tomasevic I, Remize F, Barba FJ. Lactic acid fermentation as a useful strategy to recover antimicrobial and antioxidant compounds from food and by-products. Curr Opin Food Sci. 2022;43:189–98.
7. Fachriyah E, Haryanto IB, Kusrini D, Sarjono PR, Ngadiwiyana N. Antioxidant Activity of Flavonoids from Cassava Leaves (Manihot esculenta Crantz). J Kim Sains dan Apl. 2023;26(1):10–8.
8. Ahamad A, Ansari SH. a Review on Multipurpose Medicinal Properties of Traditionally Used Psidium Guajava Leaves. Asian J Pharm Clin Res. 2022;15(8):9–22.
9. Damarhati LA, Fajriansyah MR, Anyalirria, Putra RP, Liwanda N, Nurcholis W. Optimization of Extraction Solvent for Analysis of Total Phenolic and Antioxidant Activity of Guava Leaves (Psidium guajava) using Simplex Centroid Design. J Jamu Indones. 2023;8(3):90–6.
10. Yusuf AL, Nugraha D, Indriastuti M, KR SR, Fadila LT. Physical and Activity Activity Toner of Essential Oil of Bay Leaf (Syzygium Polyanthum) Against Propionibacterium Acne. Med Sains Junal Ilm Kefarmasian. 2024;9(2):393–8.
11. Kapoor A, Sutar PP. Finish drying and surface sterilization of bay leaves by microwaves. 2019;(September):11–4.
12. Němečková I, Dragounová H, Pechačová M, Rysova J, Roubal P. Fermentation of vegetable substrates by lactic acid bacteria as a basis of functional foods. Czech J Food Sci. 2012;29(Special Issue):S42–8.
13. Sharma R, Diwan B, Singh BP, Kulshrestha S. Probiotic Fermentation of Polyphenols: Potential Sources of Novel Functional Foods. Food Prod Process Nutr. 2022;4(1).
14. Sarıtaş S, Portocarrero ACM, Miranda JM, Lombardo M, Koch W, Raposo A, et al. The

Impact of Fermentation on the Antioxidant Activity of Food Products. Molecules. 2024;29(16):3941.
15. Sharma H, Ozogul F, Bartkiene E, Rocha JM. Impact of lactic acid bacteria and their metabolites on the techno-functional properties and health benefits of fermented dairy products. Crit Rev Food Sci Nutr. 2023;63(21):4819–41.
16. Tulashie SK, Amenakpor J, Atisey S, Odai R, Akpari EEA. Production of coconut milk: A sustainable alternative plant based milk. Case Stud Chem Environ Eng. 2022;6(1):1–8.
17. Purba C, Sinaga H, Nurminah M. Effect of Ratio of Moringa Leaves Juice with Pineapple Juice and Arabic Gum on the Quality of Jelly Candy. Indones J Agric Res. 2018;1(2):162–71.
18. Xiao Y, Wang L, Rui X, Li W, Chen X, Jiang M, et al. Enhancement of the antioxidant capacity of soy whey by fermentation with Lactobacillus plantarum B1–6. J Funct Foods. 2015;12:33–44.
19. Qadi WSM, Mediani A, Benchoula K, Wong EH, Misnan NM, Sani NA. Characterization of Physicochemical, Biological, and Chemical Changes Associated with Coconut Milk Fermentation and Correlation Revealed by 1H NMR-Based Metabolomics. Vol. 12, Foods. 2023.
20. Matela KS, Pillai MK, Thamae T. Evaluation of pH, titratable acidity, syneresis and sensory profiles of some yoghurt samples from the kingdom of Lesotho. Food Res. 2019;3(6):693–7.
21. Hidayat SA, Susilo A, Masyithoh D. Effect of change in moisture content of Sumatra Forest Honey on total sugar, electrical conductivity and color. IOP Conf Ser Earth Environ Sci. 2021;788(1):12107.
22. Hanum Z, Yurliasni Y, Seutia FH. Antioxidant Activity of Fermented Goat's Milk with the Use of Bifidobacterium longum. 20 (ITAPS 21). 2022;20(Itaps 2021):374–80.
23. Elzuhria A N, Kaffah NS, N NR, Hanidah U, S AM, Nabilla FA, et al. Antibiotics Sensitivity Test Diffusion and Dilution Methods. J Res Pharm Pharm Sci. 2023 Jun;2(1 SE-ARTICLE):38–47.
24. Han CE, Ewe JA, Kuan CS, Yeo SK. Growth characteristic of probiotic in fermented coconut milk and the antibacterial properties against Streptococcus pyogenes. J Food Sci Technol. 2022 Sep;59(9):3379–86.
25. Mauro CSI, Garcia S. Coconut milk beverage fermented by Lactobacillus reuteri: optimization process and stability during refrigerated storage. J Food Sci Technol. 2019 Feb;56(2):854–64.
26. Vitheejongjaroen P, Phettakhu P, Arsayot W, Taweechotipatr M. The Ability of Lacticaseibacillus paracasei MSMC 36-9 Strain with Probiotic Potential to Ferment Coconut Milk and Produce a. 2024;
27. Magwaza LS, Opara UL. Analytical methods for determination of sugars and sweetness of horticultural products—A review. Sci Hortic (Amsterdam). 2015;184:179–92.
28. Pushpalatha N, Sreeja V, Karthik R, Saravanan G. Total Dissolved Solids and Their Removal Techniques. Int J Environ Sustain Prot. 2022;2(2):13–20.
29. Yuliana N, Koesoemawardani D, Susilawaty SA, Kurniati Y. Lactic Acid Bacteria During Fish Fermentation (Rusip). Moj Food Process Technol. 2018;6(2).
30. Yang F, Chen C, Ni D, Yang Y, Tian J, Li Y, et al. Effects of Fermentation on Bioactivity and the Composition of Polyphenols Contained in Polyphenol-Rich Foods: A Review.

Vol. 12, Foods. 2023.
31. Kumar M, Tomar M, Amarowicz R, Saurabh V, Nair MS, Maheshwari C, et al. Guava (Psidium guajava L .) Leaves : Nutritional Composition. Foods. 2021;10(752):1–20.
32. Mohidin SRNSP, Moshawih S, Hermansyah A, Asmuni MI, Shafqat N, Ming LC. Cassava (Manihot esculenta Crantz): A Systematic Review for the Pharmacological Activities, Traditional Uses, Nutritional Values, and Phytochemistry. J Evidence-Based Integr Med. 2023;28.
33. Shukla R, Jain N, Pandey V. Unlocking the potential of bay leaf: exploring its role as a nutraceutical carrier through ethnomedicinal and pharmacological insights. 2023;(January).
34. Várady M, Tauchen J, Klouček P, Popelka P. Effects of Total Dissolved Solids, Extraction Yield, Grinding, and Method of Preparation on Antioxidant Activity in Fermented Specialty Coffee. Fermentation. 2022;8(8).
35. Hidalgo-Fuentes B, Jesús-José E De, J CHA de, Sandoval-Castilla O, Teodoro E, M GRR, et al. Plant-Based Fermented Beverages: Nutritional Composition, Sensory Properties, and Health Benefits. Foods. 2024;13(6):844.
36. Špeťuch V, Petrík J, Grambalova E, Medved D, Palfy P. The capability of the viscosity measurement process. Acta Metall Slovaca. 2015 Mar;21.
37. Gilés-Gómez M, García JGS, Matus V, Quintana IC, Bolívar F, Escalante A. In Vitro and in Vivo Probiotic Assessment of Leuconostoc Mesenteroides P45 Isolated From Pulque, a Mexican Traditional Alcoholic Beverage. Springerplus. 2016;5(1).
38. Moreno-León GR, Ávila-Reyes S V, Villalobos-Espinosa JC, Camacho-Díaz BH, Tapia-Maruri D, Jiménez–Aparicio AR, et al. Effect of Agave Fructans on Changes in Chemistry, Morphology and Composition in the Biomass Growth of Milk Kefir Grains. Microorganisms. 2023;11(6):1570.
39. D'Alessandro AG, Di Luca A, Desantis S, Martemucci G. Antioxidant Synergy in a Mixture of Powder Plant Leaves and Effects on Metabolic Profile, Oxidative Status and Intestinal Morpho-Histochemical Features of Laying Hens. Animals. 2025;15(3).
40. Möwes M, Kandanda GK, Nangolo LN, Shafodino FS, Mwapagha LM. Qualitative phytochemical profiling, and in vitro antimicrobial and antioxidant activity of Psidium guajava (Guava). PLoS One. 2025;20(4 April):1–19.
41. Sulieman NG, Mohamed FK, Ibrahim MT, Ibrahim AA, Mohamed GF, Fouad MT. Bioactivity of Ethanolic Leaf Extract for Pomegranate, Guava and Green Garlic as Antioxidant and Antimicrobial Agents. Egypt J Chem. 2024;67(9):297–308.
42. De Pasquale I, Verni M, Verardo V, Gómez-Caravaca AM, Rizzello CG. Nutritional and Functional Advantages of the Use of Fermented Black Chickpea Flour for Semolina-Pasta Fortification. Vol. 10, Foods. 2021.
43. Gutierrez-Montiel D, Guerrero-Barrera AL, Chávez-Vela NA, Avelar-Gonzalez FJ, Ornelas-García IG. Psidium guajava L.: From byproduct and use in traditional Mexican medicine to antimicrobial agent. Front Nutr. 2023;10:1108306.
44. Dewi NR, Huang HT, Wu YS, Liao ZH, Lin YJ, Lee PT, et al. Guava (Psidium guajava) leaf extract enhances immunity, growth, and resistance against Vibrio parahaemolyticus in white shrimp Penaeus vannamei. Fish Shellfish Immunol. 2021;118:1–10.
45. Huynh HD, Nargotra P, Wang H min D, Shieh C jen, Liu Y chuan, Kuo C hung. Bioactive Compounds from Guava Leaves (Psidium guajava L .): Characterization , Biological Activity , Synergistic Effects , and Technological Applications. 2025;

46. Leksono BY, Cahyanto MN, Rahayu ES, Yanti R, Utami T. Enhancement of Antioxidant Activities in Black Soy Milk through Isoflavone Aglycone Production during Indigenous Lactic Acid Bacteria Fermentation. Fermentation. 2022;8(7).
47. Guadamuro L, Flórez AB, Alegría Á, Vázquez L, Mayo B. Characterization of Four B-Glucosidases Acting on Isoflavone-Glycosides From Bifidobacterium Pseudocatenulatum IPLA 36007. Food Res Int. 2017;100:522–8.
48. Alegría Á, Delgado S, Guadamuro L, Flórez AB, Felis GE, Torriani S, et al. The Genome of Bifidobacterium Pseudocatenulatum IPLA 36007, a Human Intestinal Strain With Isoflavone-Activation Activity. Gut Pathog. 2014;6(1).
49. Pacheco-Ordaz R, Antunes-Ricardo M, Gutiérrez-Uribe JA, González-Aguilar GA. Intestinal Permeability and Cellular Antioxidant Activity of Phenolic Compounds From Mango (Mangifera Indica Cv. Ataulfo) Peels. Int J Mol Sci. 2018;19(2):514.
50. Biswas BK, Rogers KG, McLaughlin F, Daniels D, Yadav AK. Antimicrobial Activities of Leaf Extracts of Guava (<i>Psidium Guajava</I>L.) on Two Gram-Negative and Gram-Positive Bacteria. Int J Microbiol. 2013;2013:1–7.
51. Zhang D, Wang Q, Li Z, Shen Z, Tan B, Zhai X. Changing the polyphenol composition and enhancing the enzyme activity of sorghum grain by solid-state fermentation with different microbial strains. J Sci Food Agric. 2024 Aug;104(10):6186–95.
52. Liu S, He Y, He W, Song X, Peng Y, Hu X, et al. Exploring the Biogenic Transformation Mechanism of Polyphenols by Lactobacillus plantarum NCU137 Fermentation and Its Enhancement of Antioxidant Properties in Wolfberry Juice. J Agric Food Chem. 2024 Jun;72(22):12752–61.
53. Birmeta G, Bakeeva A, Passoth V. Yeasts and bacteria associated with kocho, an Ethiopian fermented food produced from enset (Ensete ventricosum). Antonie van Leeuwenhoek, Int J Gen Mol Microbiol. 2019;112(4):651–9.
54. Aguirre-Garcia YL, Nery-Flores SD, Campos-Muzquiz LG, Flores-Gallegos AC, Palomo-Ligas L, Ascacio-Valdés JA, et al. Lactic Acid Fermentation in the Food Industry and Bio-Preservation of Food. Fermentation. 2024;10(3).
55. LA A, F.S I, I A. Production and Characterization of Bacteriocin from Lactic Acid Bacteria Isolated from Fermented Food Products. 2024;23(3):101–14.