



Research Article

# Impact of lactic acid fermentation using *Lactiplantibacillus plantarum* on the physicochemical, phytochemical, and biological properties of *Antidesma bunius* (L.) Spreng. fruit juice

Jerald P. Nituda<sup>†</sup>, Rona Camille M. Lizardo-Agustin<sup>†\*</sup>

*Institute of Food Science and Technology, College of Agriculture and Food Science, University of the Philippines Los Baños, College, Laguna 4031, Philippines*

**Abstract** Lactic acid fermentation is a transformative method, converting polyphenols in plant-based juices into bioactive derivatives. *Antidesma bunius* (L.) Spreng. fruit, with its high polyphenolic content, presents an opportunity for innovative functional food products. In this study, changes in physicochemical properties, polyphenolic contents, and *in vitro* biological activities of lactic acid-fermented *A. bunius* fruit juice were investigated. Fermentation by *Lactiplantibacillus plantarum* resulted in significant changes in the physicochemical, phytochemical, and biological properties of *A. bunius* juice. The initial decline and subsequent increase in LAB count aligned with changes in total soluble solids, pH, and total titratable acidity, indicating lactic acid production. Total polyphenols were retained after 48 h, while the total flavonoids increased after 72 h fermentation. The antioxidant activities in terms of H<sub>2</sub>O<sub>2</sub> scavenging and Fe<sup>3+</sup> reducing power increased at 24 h and 48 h, respectively. Alpha-amylase inhibition peaked at 48 h fermentation, while  $\alpha$ -glucosidase inhibition declined but remained significant. HPLC analysis showed that gallic acid and catechin were the specific bioactive compounds that significantly increased after fermentation. Chemometric analysis showed that gallic acid and catechins had the most positive effect in the improvement of biological activities specifically  $\alpha$ -amylase and  $\alpha$ -glucosidase inhibition, and H<sub>2</sub>O<sub>2</sub> scavenging activities. The study revealed that lactic acid fermentation of *A. bunius* fruit juice enhanced its physicochemical properties, polyphenolic content, and specific biological activities.



OPEN ACCESS

**Citation:** Nituda JP, Lizardo-Agustin RCM. Impact of lactic acid fermentation using *Lactiplantibacillus plantarum* on the physicochemical, phytochemical, and biological properties of *Antidesma bunius* (L.) Spreng. fruit juice. Food Sci. Preserv., 31(5), 721-734 (2024)

**Received:** July 01, 2024  
**Revised:** September 03, 2024  
**Accepted:** September 05, 2024

**Keywords** functional food, lactic acid fermentation, *Antidesma bunius* L. Spreng. fruit, polyphenols, bioactivity

<sup>†</sup>These authors contributed equally to this study.

**\*Corresponding author**  
Rona Camille M. Lizardo-Agustin  
Tel: +63-917-163-8718  
E-mail: rmlizardo@up.edu.ph

Copyright © 2024 The Korean Society of Food Preservation. This is an Open Access article distributed under the terms of the Creative Commons Attribution Non-Commercial License (<http://creativecommons.org/licenses/by-nc/4.0>) which permits unrestricted non-commercial use, distribution, and reproduction in any medium, provided the original work is properly cited.

## 1. Introduction

In recent years, the health landscape has notably evolved, emphasizing the improvement of nutrition by changing dietary habits (Chopra et al., 2022). Health-conscious consumers are increasingly opting for food products with the aim of preventing negative health consequences, particularly chronic diseases such as cancer and diabetes. This trend has given rise to the functional foods market, which are products containing bioactive constituents to maintain optimum human health (Tsoupras et al., 2023). Innovations have contributed to significant market growth, reaching its peak during the COVID-19 pandemic (Fortune Business Insights, 2019; Lordan, 2021). Moreover, consumers are actively seeking beverages that offer enhanced functionality, leading to a surge in demand for functional beverages with innovative properties. This growing interest has propelled the market to significant value (Gonçalves et al., 2022).

Lactic acid fermentation represents a biotransformative food processing method, most especially for plant-based beverages, as it leads to enhancing health benefits (Plessas, 2021). This process significantly impacts the bioavailability of beneficial fruit juice components through the metabolic actions of lactic acid bacteria (LAB). The most frequent species of LAB involved in the fermentation of plant-based substrates, such as fruits, are *Lactiplantibacillus plantarum*, *Lb. brevis*, *Lb. rhamnosus*, *Lb. acidophilus*, *Leuconostoc mesenteroides*, *Lc. citreum*, *Lc. fallax*, *Lc. kimchi*, *Pediococcus pentosaceus*, *P. acidilactici*, *Weissella confusa*, *W. cibaria*. Interestingly, *L. plantarum*, became of great interest in research because of its enzyme systems, which can hydrolyze or produce important compounds (Septembre-Malaterre et al., 2018). One major finding showed that LAB, particularly *L. plantarum*, which is common in most plant-based fermentation, can convert polyphenols into bioactive derivatives through phenolic acid enzyme actions, resulting in enhanced bioavailability for humans (Filannino et al., 2018). LAB-fermented juices also exhibit functional properties, such as increased nutrient density through the bioconversion of sugars (Septembre-Malaterre et al., 2018), improved nutrient bioavailability (Filannino et al., 2018), and enhanced antioxidant activity (Li et al., 2019). In a similar study done by Vivek et al. (2019), the Sohiong juice fermented by *L. plantarum* exhibited an increase in the antioxidant properties and total phenolic and anthocyanin contents. Reviews also indicate the health-promoting properties of various LAB-fermented plant-based beverages (Szutowaska, 2020). These include antidiabetic, anti-inflammatory, and anticancer effects, and an impact on reducing obesity. Thus, lactic acid fermentation can be considered a strategic method for food supplementation in addressing chronic diseases and obesity.

The *Antidesma bunius* (L.) Spreng. (bignay) tree, which bears clusters of small fruits, has a genus with 150 species distributed across Asia, Africa, Australia, and the Pacific Islands (Crieta et al., 2022; Islam, 2018). It is known as *bignay* or *bugnay* (Philippines), *buni* or *berunai* (Malaya, India), *wooni* or *hooni* (Indonesia), *ma mao luang* (Thailand), *kho lien tu* (Laos), *choi moi* (Vietnam), and *moi-kin* or *chunka* (natives in Queensland). The ripe fruits are commonly consumed directly, although their uptake is limited due to the tart taste and potential for deep purple pigment stains upon consumption (Carbonera, 2023). To enhance their appeal and usability, various processing methods, such as sugar concentration,

have been employed to produce jams, jellies, and preserves. Additionally, fruit concentrates are frequently utilized to produce tropical fruit wine through alcoholic fermentation and ready-to-drink juice through pasteurization (Gestupa, 2015). The existing *A. bunius* fruit products play a crucial role in commercialization, contributing to economic value, extending shelf life, and enhancing nutritional content.

The mature fruits exhibit rich polyphenolic content, notably from two of the major families, namely phenolic acids and flavonoids (Gestupa et al., 2016; Sartagoda et al., 2021). Among the flavonoids, anthocyanins contribute significantly to the vibrant color of the fruit and play a significant role during fermentation (Palencia-Argel et al., 2022). Ripe *A. bunius* concentrate has been identified to possess high levels of total polyphenols and anthocyanins (Hardinasinta, 2021). Known for their potent antioxidant activities, these polyphenols are associated with a potential risk reduction in cancer (Zubia, et al., 2023). Additionally, *in vivo* studies involving the fruit have demonstrated a notable decrease in blood glucose levels (Udomkasemsabet et al., 2019), suggesting a potential link to reduced risk of diabetes.

The objective of this study is to evaluate the changes in the physicochemical properties, polyphenolic contents, and biological activities of *A. bunius* fruit juice as influenced by lactic acid fermentation. This utilization represents an approach to investigate the enhancement of nutritional and health-promoting effects, based on changes in the amounts of polyphenols and biological activities, towards the development of innovative functional food products and ingredients for nutrition and health.

## 2. Materials and methods

### 2.1. *Antidesma bunius* (L.) Spreng. juice preparation

The ripe fruits of *A. bunius* ([L.] Spreng var. Common) were procured from locations in Laguna, Philippines, and transported to the Institute of Food Science and Technology (IFST), UP Los Baños. After a thorough washing under running tap water to eliminate any contaminants, the fruits were placed in polyethylene (PE) bags and stored in the freezer until use. Upon thawing, the fruits were homogenized using a commercial blender. The puree was then filtered to separate the pulp from the juice (yield: 81.82%; total soluble solids: 7 °Brix; pH 2.93).

For juice preparation, concentrations of 25%, 50%, and 75% v/v were made by dilution with distilled water. The total soluble solids (TSS) of the samples were adjusted to 12 °Brix while the pH was adjusted to 4.0 with food-grade sodium bicarbonate. Rapid pasteurization as applied in acid or acidified beverages such as fruit juices was done by heating 1 L of each juice sample to 85°C for 1 min. Pasteurization temperature was reached after 7 min. The juice samples were allowed to cool to 37°C in a thermostatically controlled water bath prior to inoculation with lactic acid bacteria.

## 2.2. Lactic acid fermentation

A pure culture of *L. plantarum* BIOTECH 1223 (Philippine National Collection of Microorganisms, Philippines) was used as a starter culture for *A. bunius* juice fermentation, which was cultured in sterile De Man, Rogosa, and Sharpe (MRS) broth (Biokar Diagnostics, Allonne, France). The pure culture was incubated with shaking (BJPX-2102D, Biobase, Shandong, China) at 37°C and 100 rpm for 16 h. Consequently, the *L. plantarum* cells were washed twice using a sterile 0.1% saline solution at 2,739 ×g for 20 min. The cells were then aseptically inoculated to the prepared *A. bunius* juices at the rate of 1% of the volume of the juice. The juice samples were incubated with shaking at 37°C and 100 rpm for up to 96 h. Sampling was done every 24 h for analysis. At each sampling point, the juice samples were pasteurized at 85°C for 1 min to ensure non-interference of the bacteria in the analysis.

## 2.3. Phytochemical content analysis

The analysis of total polyphenol content (TPC) employed the Folin-Ciocalteu colorimetric method (Lizardo et al., 2020), with absorbance measured at 760 nm using a 96-well plate microplate reader (BK-EL10C, Biobase). The results were quantified in terms of mg gallic acid equivalents (GAE) per mL sample. Meanwhile, the analysis of total flavonoid content (TFC) employed the aluminum chloride colorimetric method (Fattahi et al., 2014) with few modifications. Absorbance was measured at 492 nm using a microplate reader, and the results were expressed as mg quercetin equivalents (QE) per mL sample. The total monomeric anthocyanin content was determined through a modified pH differential method (Giusti and Wrolstad, 2001). Twenty µL of prepared sample were mixed separately with 100 µL potassium chloride buffer (pH

1.0, 0.025 M) and 100 µL potassium acetate buffer (pH 4.5, 0.4 M). The mixtures were held at room temperature for 20 min. The absorbances of separate mixtures were measured using a microplate reader at 520 nm and 700 nm. The TAC was computed using Equation (1) and expressed as mg cyanidin-3-glucoside equivalents (C3GE) per L sample.

$$\text{Anthocyanin pigment (mg C3GE/L)} = \frac{A \times MW \times DF \times 10^3}{\epsilon \times l} \quad (1)$$

Where  $A$  is the absorbances  $(A_{520\text{nm}} - A_{700\text{nm}})_{\text{pH } 1.0} - (A_{520\text{nm}} - A_{700\text{nm}})_{\text{pH } 4.5}$ ,  $MW$  is the molecular weight of cyanidin-3-glucoside (449.2 g/mol),  $DF$  is the dilution factor,  $\epsilon$  is the molar extinction coefficient of cyanidin-3-glucoside (26,900 L mol<sup>-1</sup> cm<sup>-1</sup>),  $l$  is the cell pathlength for microplate reader (0.3 cm), and  $10^3$  is the conversion factor from g to mg.

Based on the result of the total phytochemical content screening, the selected concentration and fermentation time were 75% v/v and 0-72 h, respectively.

## 2.4. Monitoring of physicochemical properties during lactic acid fermentation

*L. plantarum* count was evaluated every 24 h through the standard plate count method (American Public Health Association, 2018) using MRS agar as the culture medium. Subsequently, the pH, TSS, and total titratable acidity (TTA) of the juice samples were determined. The TSS was measured using a refractometer. The pH was measured using a benchtop pH meter (AB33PH-B, OHAUS Instruments, Shanghai, China). The TTA was measured using the method employed by Tyl and Sadler (2017) and was calculated using Equation (2):

$$\text{TTA (\% lactic acid w/v)} = \frac{N \times V1 \times Eq. Wt.}{V2 \times 10} \quad (2)$$

Where  $N$  is the normality of NaOH (mEq/mL);  $V1$  is the volume of NaOH (mL);  $Eq. wt.$  is the equivalent weight of lactic acid (90.08 mg/mEq);  $V2$  is the volume of sample (mL).

## 2.5. Polyphenolics profiling using HPLC

The method parameters employed in this study were adapted from Flandez et al. (2023) and utilized a High-

Performance Liquid Chromatography (HPLC) system (Shimadzu, Tokyo, Japan), as described in the same author's work. Before conducting the HPLC analysis, the samples were prepared through a 25-fold dilution process. This involved combining 1 gram of the sample with 25 mL of an extractant solution comprising a 50% methanol solution (RCI Labscan, Bangkok, Thailand) acidified with 1% glacial acetic acid (RCI Labscan). The quantification was determined using standard curves derived from the adopted method, and concentrations were expressed as mg polyphenol per mL sample.

## 2.6. Assessment of biological activities

### 2.6.1. Antioxidant activities

The antioxidant activities of the juice samples were analyzed in comparison to a 0.1% solution of the butylated hydroxyanisole (BHA) standard (Sigma-Aldrich, Darmstadt, Germany). This involved evaluations of free radical scavenging, reactive oxygen species (ROS) scavenging, and reducing power. Free radical scavenging activities were assessed using 1,1-diphenyl-2-picrylhydrazyl (DPPH<sup>•</sup>) radical (Loba Chemie, Mumbai, India) and 2,2'-Azino-bis(3-ethylbenzothiazoline-6-sulfonate) diammonium salt (ABTS<sup>•+</sup>) radical (Sigma-Aldrich). The protocols by Lizardo et al. (2020) and González-Palma et al. (2016) were followed for DPPH<sup>•</sup> and ABTS<sup>•+</sup> scavenging activities, respectively. Additionally, ROS scavenging involving hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) employed the procedure outlined by Lizardo et al. (2020). The absorbances for the DPPH<sup>•</sup>, ABTS<sup>•+</sup>, and H<sub>2</sub>O<sub>2</sub> assays were measured at 540, 700, and 405 nm, respectively, using a microplate reader. The scavenging activities, expressed as percentages, were calculated using Equation (3).

$$\text{Scavenging activity (\%)} = \frac{A_0 - A_1}{A_0} \times 100 \quad (3)$$

Where  $A_0$  is the absorbance of the blank, and  $A_1$  is the absorbance of the sample.

Concurrently, the reducing power of the samples was assessed by adopting the method employed by Lizardo et al. (2020). The absorbance values were measured at 700 nm using a microplate reader. The reducing power, determined through the reduction of Fe<sup>3+</sup> (FRAP Value), was computed<sup>31</sup> using Equation (4).

$$\text{Reducing power (FRAP value)} = \frac{A_1 - A_0}{A_C - A_0} \times 2 \quad (4)$$

Where  $A_1$  is the absorbance of the sample,  $A_0$  is the absorbance of the blank, and  $A_C$  is the absorbance of the control (0.1% BHA standard).

### 2.6.2. Enzyme-inhibition activities

The inhibitory effects on  $\alpha$ -amylase (derived from *Aspergillus oryzae*, Sigma-Aldrich) and  $\alpha$ -glucosidase (derived from *Saccharomyces cerevisiae*, Sigma-Aldrich) were evaluated using the selected fermented samples, with 0.1% w/v acarbose (Tokyo Chemical Industry, Tokyo, Japan) employed as the standard enzyme inhibitor. The  $\alpha$ -amylase inhibitory activity assay was based on the methodology by Kazeem et al. (2013) with slight modification. Briefly, 250  $\mu$ L of the prepared sample was combined with 250  $\mu$ L of  $\alpha$ -amylase solution (0.5 mg  $\alpha$ -amylase powder per mL 0.02 M sodium phosphate buffer pH 6.9). After pre-incubation for 10 minutes at room temperature, 250  $\mu$ L of a 1% solution of soluble starch (Loba Chemie) was added as the substrate. The reaction mixture was incubated for an additional 10 minutes at room temperature. Following this, 96 mM of 3,5-Dinitrosalicylic acid (DNS) coloring reagent was introduced, and the mixture was incubated in an 80°C water bath for 5 minutes. The cooled mixture was then transferred to a 96-well plate, and absorbance was measured at 492 nm. The % inhibition activity was calculated (Lankatillake et al., 2021; Sun et al., 2024) using Equation (5).

Additionally, the  $\alpha$ -glucosidase inhibitory activity, adopted from Lizardo et al. (2020), involved an absorbance assay with *p*-Nitrophenyl  $\alpha$ -D-glucopyranoside (*p*-NPG) (Sigma-Aldrich) as the substrate, measured at 405 nm in microplate reader to determine *p*-nitrophenol. The % inhibition activity was computed using Equation (6). The percentage inhibition of the samples was standardized by normalizing it against the percentage inhibition obtained with the acarbose standard (Lankatillake et al., 2021; Sun et al., 2024).

$$\alpha\text{-Amylase inhibition (\%)} = 1 - \left( \frac{B-b}{A} \right) \times 100 \quad (5)$$

$$\alpha\text{-Glucosidase inhibition (\%)} = 1 - \left( \frac{B-(b+A)}{A-a} \right) \times 100 \quad (6)$$

Where  $A$  is the absorbance of the reaction mixture containing enzyme solution but no sample,  $a$  is the absorbance without enzyme solution and sample,  $B$  is the absorbance containing enzyme solution and sample, and  $b$  is the absorbance containing sample but no enzyme solution.

## 2.7. Statistical analysis

The analyses were conducted in triplicate, and the results were expressed as mean±standard deviation. Microsoft® Excel for Mac Version 16.81 (24011420) (Microsoft, Redmond, WA, USA) was employed to generate the computed dataset and graphs. Using *jamovi* Version 2.3.3 (The *jamovi* project, New South Wales, Australia), Analysis of Variance (ANOVA) was initially conducted, followed by Tukey's test ( $p < 0.05$ ), to identify significant differences among variables (fermentation time and juice dilution levels). Post-hoc pairwise comparisons of all treatment means were carried out using the software developed by Assaad et al. (2015).

A chemometric evaluation was performed using RStudio (Posit Software, Boston, MA, USA) Version 2023.06.1+524. The assessments comprised Pearson's correlation matrix and Principal Component Analysis (PCA) biplot to elucidate the relationships among the polyphenolic contents of the fermented samples and their biological activities.

## 3. Results and discussion

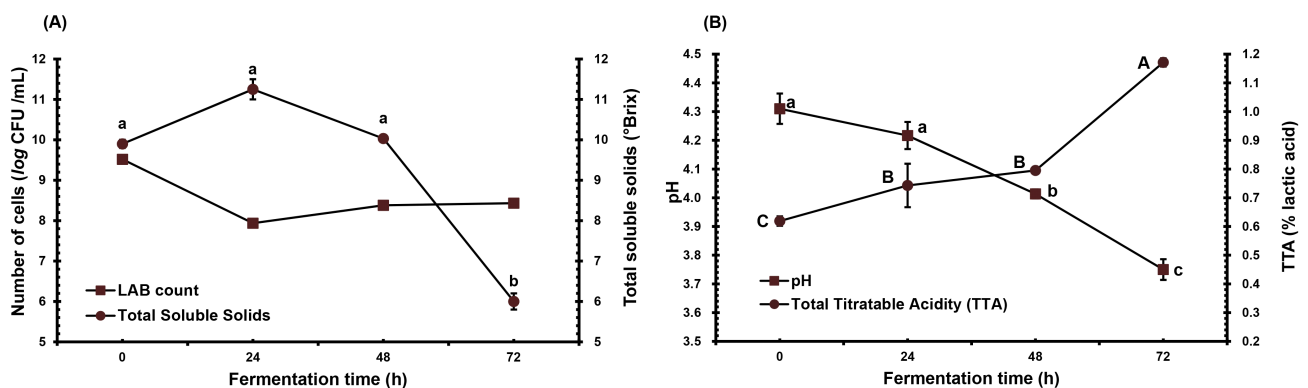
### 3.1. Changes in physicochemical properties

The physicochemical properties throughout the fermentation

process were monitored to determine the effect of the process at the specified time period. Fig. 1A and 1B presents the changes in *L. plantarum* count, TSS, pH, and over fermentation time until 72 h. The cell population (Fig. 1A) initially decreased by 1.5 log cycles after 24 h (7.94 log CFU per mL), followed by an increase in the 48th and 72nd hour (8.38-8.43 log CFU per mL) of fermentation. Nonetheless, the *L. plantarum* count towards the end of the fermentation was lower than the initial count.

Meanwhile, a significant drop in TSS by 4 °Brix after 72 h of fermentation was observed ( $p < 0.05$ ). Fig. 1B illustrates a decrease in pH from 4.3 to 3.8 and a significant increase in TTA from 0.62% to 1.17% during the 72-hour fermentation period ( $p < 0.05$ ), indicating the production of organic acids, primarily lactic acid (Tian et al., 2019).

The growth of *L. plantarum* during fermentation aligns with findings in different studies, showing a sharp decline (Mustafa et al., 2019) and a subsequent recovery (Fazeli et al., 2011). The initial drop in cell count could be attributed to the adjustment phase of the microorganisms as the substrate contains high amounts of phenolics and organic acids, which may be detrimental to their growth (Fazeli et al., 2011; Mousavi et al., 2011; Nualkaekul et al., 2012; Sheehan et al., 2007) and potentially leading to changes in the membrane fatty acid composition of *L. plantarum* (Rozès and Peres, 1998). The subsequent increase in the bacterial count is influenced by the prebiotic properties of anthocyanin and its monomer (Zhu et al., 2018) *A. bunius* fruit concentrate, known for its high anthocyanin content (Hardinasinta et al., 2021), provides these compounds that promote increased



**Fig. 1.** Changes in the physicochemical properties of *Antidesma bunius* juice during lactic acid fermentation. *Lactiplantibacillus plantarum* growth and total soluble solids (A) and pH and total titratable acidity (B). All values are mean±SD ( $n=3$ ). Different letters (<sup>a-c</sup>, <sup>A-C</sup>) on the bars indicate significant differences across fermentation times ( $p < 0.05$ ).

LAB viability. The viability of *L. plantarum* throughout the fermentation can be associated with the decrease in TSS and pH and the increase in TTA, possibly resulting from bacterial metabolism of the fruit's significant secondary metabolites, such as anthocyanin (Palencia-Argel et al., 2022). The catabolism produces simpler polyphenols with superior antioxidant activity compared to the precursors (Ruiz Rodríguez et al., 2021).

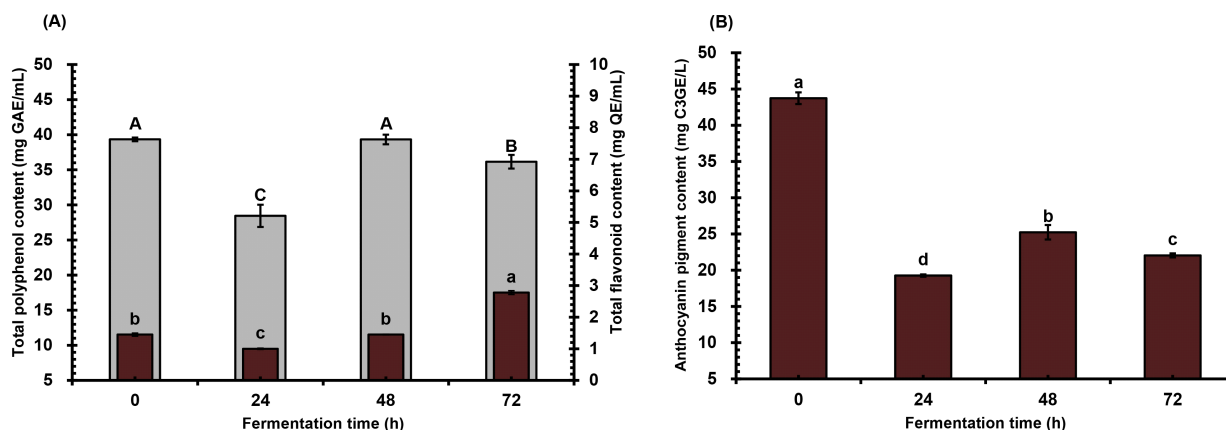
### 3.2. Changes in polyphenolic contents

To determine the effect of lactic acid fermentation by *L. plantarum* on the total phytochemical and specific phenolic contents of *A. bunius* juice over time, the total polyphenol, total flavonoid, and total anthocyanin contents were measured, and results are shown in Fig. 2. The highest and lowest total polyphenol concentrations were observed at 48 hours (39.33 mg GAE/mL) and 24 hours (28.44 mg GAE/mL) of fermentation, respectively (Fig. 2A). Meanwhile, the total flavonoids displayed an overall increasing trend, characterized by a substantial increase starting from 1.45 mg QE/mL at 48 hr fermentation period. The results show that at a particular time and point during fermentation, an improvement and possible evolution of the phenolic content of the fruit juice occur, which may differ based on the substrate and microorganisms involved. Meanwhile, Fig. 2B illustrates the dynamics of total monomeric anthocyanin content. The graph demonstrates an initial marked decrease (19.26 mg C3GE/L), succeeded by a significant increase (25.23 mg C3GE/L), and then undergoes another substantial decrease (22.04 mg C3GE/L). Notably, the anthocyanin concentrations observed during fermentation declined and are

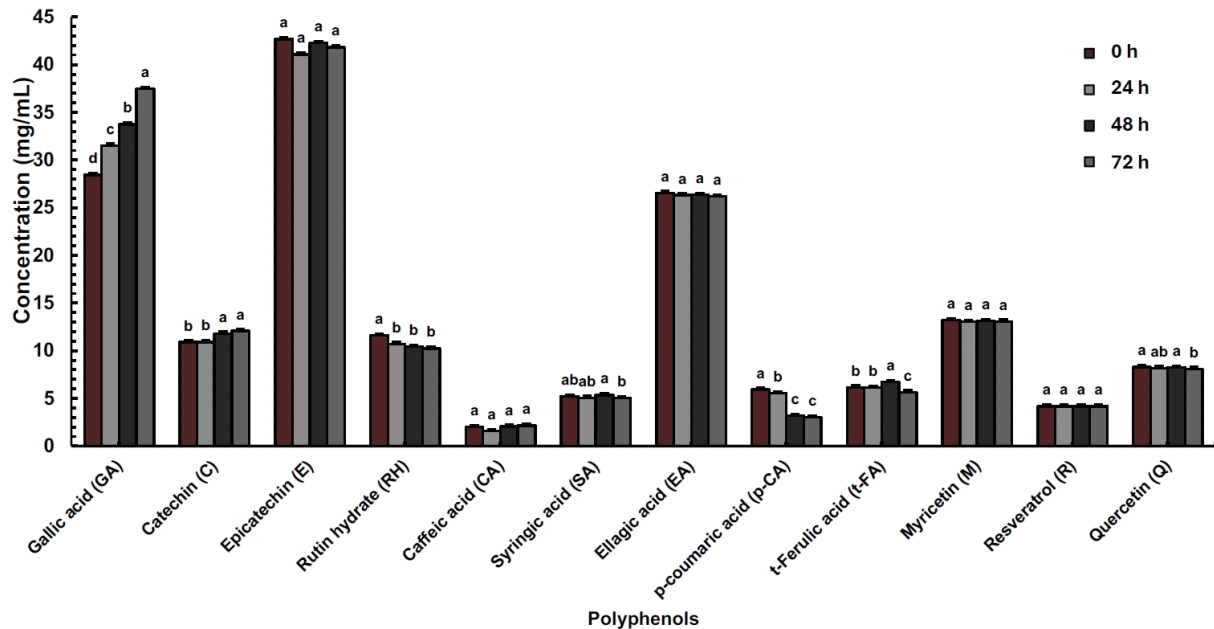
significantly lower compared to the unfermented juice sample (43.73 mg C3GE/L).

The change in the concentration of specific phenolic compounds as influenced by lactic acid fermentation was also analyzed. Fig. 3 illustrates the changes in predominant polyphenols found in horticultural crops through the fermentation of *A. bunius* fruit juice. Among the phenolic acids, there was a substantial increase in gallic acid, while a significant decrease was observed in rutin hydrate, *p*-coumaric acid, and quercetin, which started at 48 h, 24 h, and 72 h, respectively. The phenolic acids *t*-ferulic acid and syringic acid follow a similar trend, increasing at 48 h and decreasing at 72 h. Additionally, there was a substantial increase in the flavonoid catechin ( $p < 0.05$ ).

The similarity in the amount of total phenolics between the unfermented and LAB-fermented fruit juice was also previously documented (Di Cagno et al., 2011). Their lack of significant differences may imply the overall dynamic shift in the polyphenolic profile influenced by bacterial metabolism during fermentation. This includes an overall increase in total flavonoids and a reduction in total anthocyanins, phenolic acids, and flavonoids, accompanied by an elevated concentration of some phenolic acids and flavonoids. One possible bacterial mode of action is associated with anthocyanin metabolism (Palencia-Argel et al., 2022), leading to a significant decrease in total anthocyanin compared to the unfermented *A. bunius* fruit juice. Catabolizing of anthocyanins involves reactions such as hydrolysis, liberating gallic acid and syringic acid (de Llano et al., 2017), and the formation of co-pigmentation



**Fig. 2.** Total phytochemical contents of *Antidesma bunius* juice fermented by *Lactiplantibacillus plantarum* at different fermentation times. Total phenolic and flavonoid contents (A) and Total anthocyanin content (B). All values are mean±SD (n=3). Different letters (A-C, a-d) on the bars indicate significant differences ( $p < 0.05$ ).



**Fig. 3.** Polyphenol profile of *Antidesma bunius* juice fermented by *Lactiplantibacillus plantarum* at different fermentation time. All values are mean $\pm$ SD (n=3). Different letters (<sup>a-d</sup>) on the bars indicate significant differences (p<0.05).

complexes with catechin, leading to increased amounts of some polyphenols during lactic fermentation. The result confirms that the juice sample contains high amounts of polyphenols, which is supported by existing studies on *A. bunius* fruit (Recuenco et al., 2016; Sartagoda et al., 2021) and concentrate (Hardinasinta et al., 2021). Probable polyphenolic reactions include the hydrolysis and demethylation (de Llano et al., 2017) of rutin hydrate to gallic acid and quercetin (China et al., 2012), *p*-coumaric acid to *t*-ferulic acid (Liao et al., 2023, and quercetin to catechin. The increased levels of polyphenolic metabolites (i.e., gallic acid, *t*-ferulic acid, syringic acid, catechin) can be associated with improved biological activities of the juice (Jamar et al., 2017).

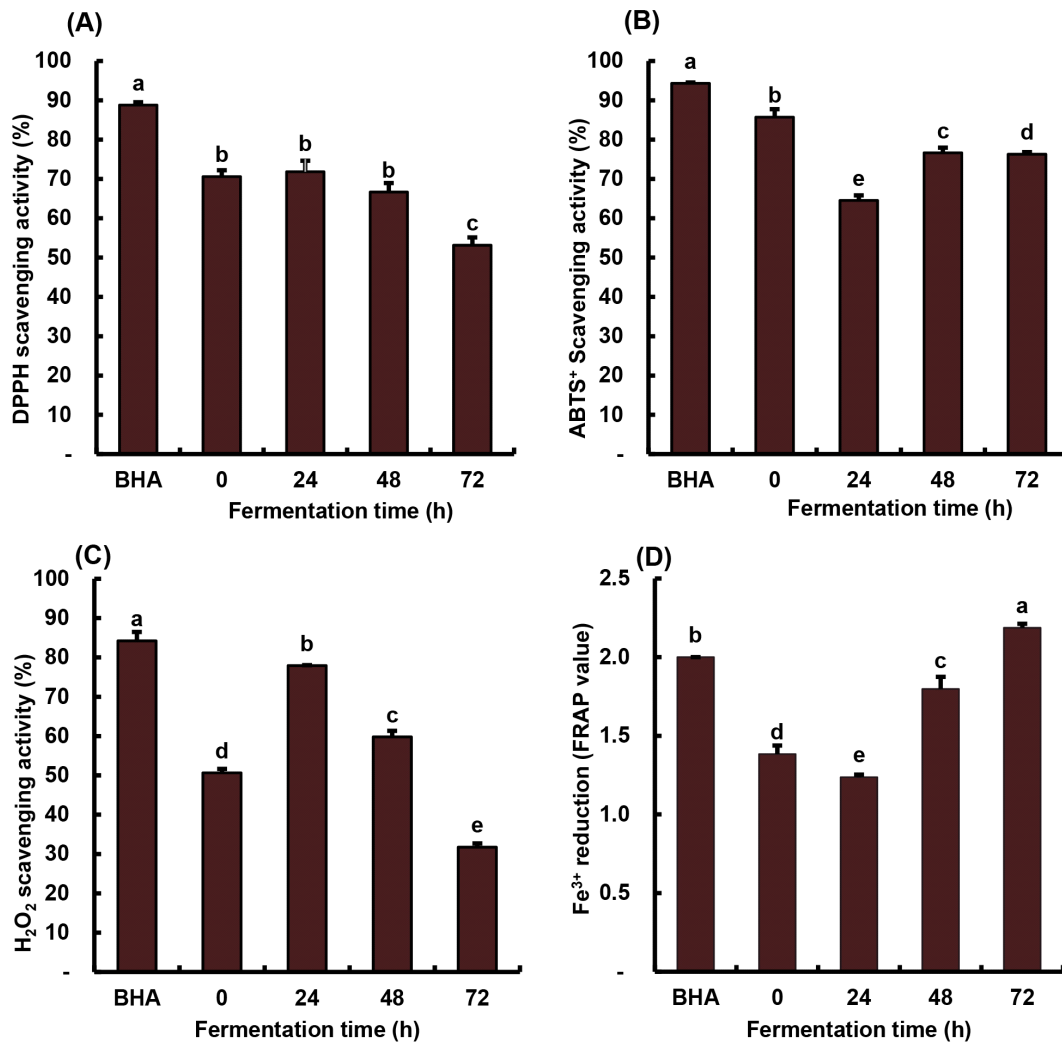
### 3.3. Changes in biological activities

#### 3.3.1. Antioxidant activities

The antioxidant activities of *A. bunius* juice were assessed to monitor changes and demonstrate the effect of lactic acid fermentation (Fig. 4). The BHA standard, a common antioxidant used in foods, showed the highest scavenging activities (84–94%) compared to the juice samples (Fig. 4A–4C) but exhibited lower reducing power than the 72-hour fermented juice (Fig. 4D). The DPPH<sup>•</sup> scavenging activity began to

decline, reaching 53% at 72 h (Fig. 4A). ABTS<sup>•+</sup> scavenging activity fluctuated during fermentation, peaking at 48 h (77%) (Fig. 4B). H<sub>2</sub>O<sub>2</sub> scavenging activity increased at 24 h (78%) and then started to decrease at 48 h (Fig. 4C). Meanwhile, the Fe<sup>3+</sup> reducing power showed an initial slight decrease in the graph, followed by a continuous and notable upward trend (Fig. 4D). Several studies have established the remarkable antioxidant activity of phenolics, primarily attributed to their ability to transfer hydrogen atoms or electrons to free radicals (Wang et al., 2022).

A study by Thbayh and Fiser (2022) presented that synthetic BHA exhibited more antioxidant potential than natural polyphenols, which is consistent with the findings of this study. Similar trends were observed in the lactic acid fermentation of *A. bunius* fruit juice for DPPH<sup>•</sup> (Di Cagno et al., 2017), ABTS<sup>•+</sup> (Fessard et al., 2016), and H<sub>2</sub>O<sub>2</sub> (Wouters et al., 2013) scavenging activities, as well as Fe<sup>3+</sup> reducing power (Yang et al., 2014) in some lactic acid fermented fruits and vegetables. In this study, ABTS<sup>•+</sup> exhibited higher reactivity than DPPH<sup>•</sup> due to the solvent environment during the assay, radical mode of synthesis, and radical stability (Durmaz et al., 2023). Meanwhile, the decreasing DPPH<sup>•</sup> and sudden initial increase in H<sub>2</sub>O<sub>2</sub> scavenging effect are attributed to the production of bacterial



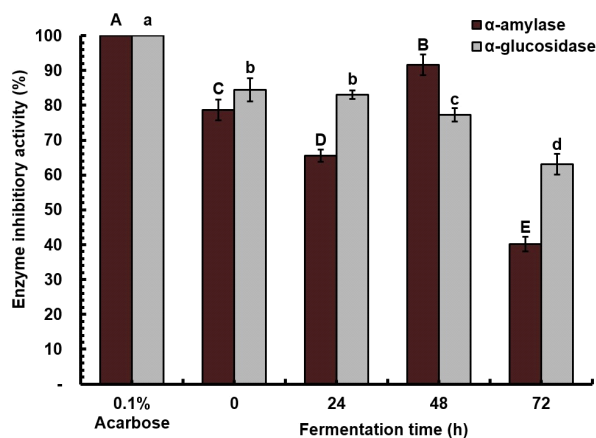
**Fig. 4.** Antioxidant activities of *Antidesma bunius* juice fermented by *Lactiplantibacillus plantarum* at different fermentation time. Assays include DPPH<sup>•</sup> scavenging activity (A), ABTS<sup>•+</sup> scavenging activity (B), H<sub>2</sub>O<sub>2</sub> scavenging activity (C), and Ferric reducing power (D). All values are mean±SD (n=3). Different letters (a-e) on the bars indicate significant differences (p<0.05).

metabolites during fermentation, which can be utilized in the assay reaction. The mechanism of reducing power involves the transfer of electrons by adding Fe<sup>3+</sup> to the antioxidant to develop Fe<sup>2+</sup>, which correlates with antioxidant capacity (Durmaz et al., 2023; Sultana et al., 2017). Juice samples with compounds that exhibit redox properties and high reducing power can serve as electron donors, preventing oxidative damage (Lizardo et al., 2020). The increase in free-form polyphenols and the generation of byproducts during lactic acid fermentation of *A. bunius* juice contribute to the improved antioxidant capacity for consistently enhancing health-promoting properties such as chronic diseases and obesity prevention (Zhao et al., 2021).

### 3.3.2. Enzyme-inhibition activities

The subsequent *in vitro* assessment of inhibiting endogenous carbohydrases ( $\alpha$ -amylase and  $\alpha$ -glucosidase) was conducted to observe changes and determine the impact of fermentation on such properties of *A. bunius* fruit juice (Fig. 5). Concerning acarbose, which exhibited the highest activity,  $\alpha$ -amylase inhibitory activity displayed a fluctuating trend during fermentation, reaching its peak at 48 hours (92%), surpassing the activity observed in the unfermented juice (77%). Conversely,  $\alpha$ -glucosidase inhibitory activity decreased progressively over the fermentation period, starting at 84%, declining to 77% at 48 hours, and eventually reaching 63%.





**Fig. 5.** Enzyme inhibitory activities ( $\alpha$ -amylase and  $\alpha$ -glucosidase) of *Antidesma bunius* juice fermented by *Lactiplantibacillus plantarum* at different fermentation time. All values are mean $\pm$ SD (n=3). Different letters (<sup>A-E</sup>, <sup>a-d</sup>) on the bars indicate significant differences (p<0.05).

Most of the compounds correlate to antioxidant activity and show a correlation with antidiabetic activity (Razola-Díaz et al., 2023).

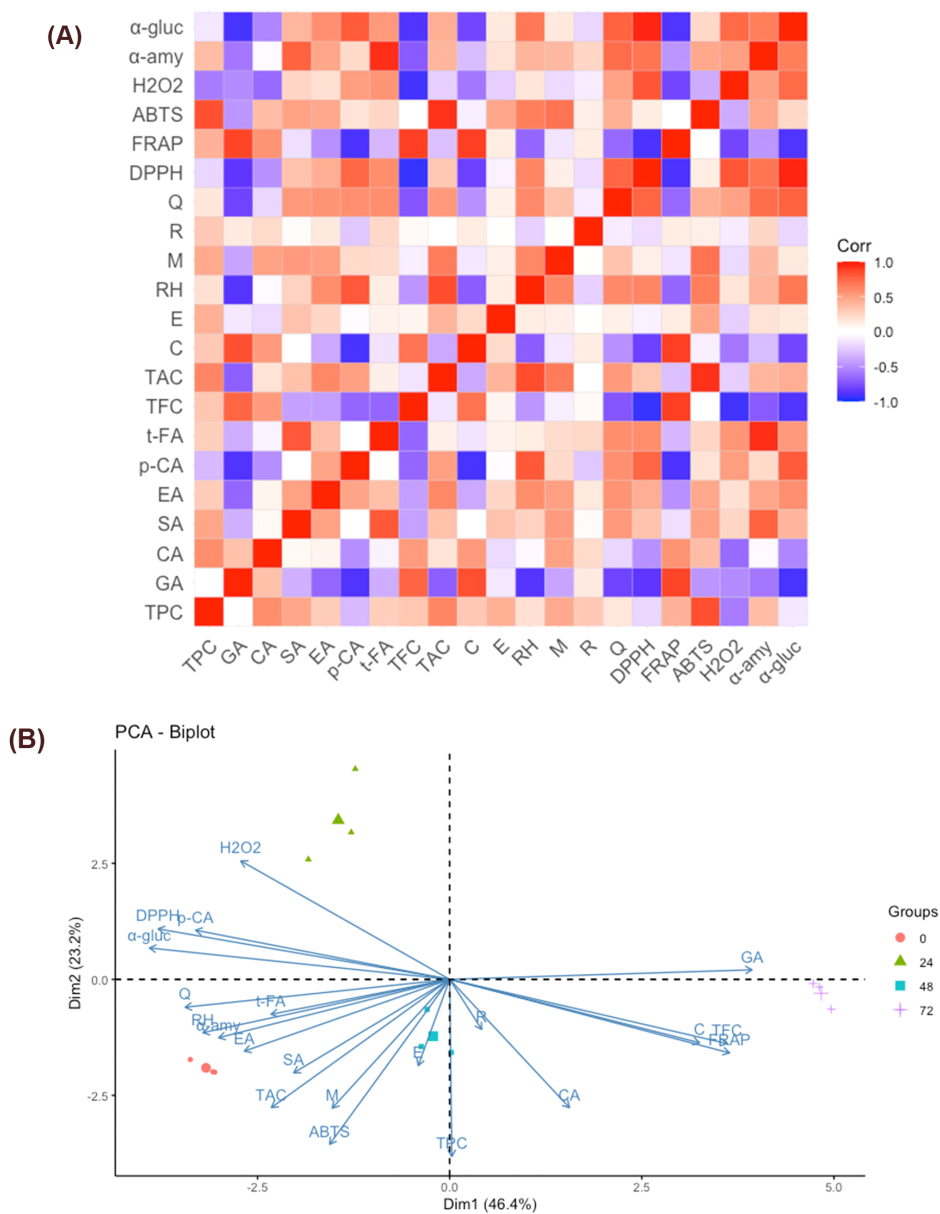
Acarbose, an enzyme inhibitor in diabetes treatment, is linked to adverse effects such as abdominal pain and flatulence, demanding a search for natural alternatives (Lankatillake et al., 2021). The observed *in vitro*  $\alpha$ -amylase inhibition aligns with the study of Razola-Díaz et al. (2023), indicating greater potency in *L. plantarum*-fermented avocado seed compared to the unfermented seed, attributed to its numerous polyphenols (Razola-Díaz et al., 2023). The fermented juice exhibited potent  $\alpha$ -glucosidase inhibitory activity, suggesting promising avenues for future research. Polyphenols, which serve as natural inhibitors for  $\alpha$ -amylase and  $\alpha$ -glucosidase, competitively bind to the active sites of enzymes, thereby inhibiting their activity (Wu et al., 2021). They also play a crucial role in glycemic control by enhancing insulin receptor sensitivity and improving glucose uptake by tissues (Isas et al., 2023). Furthermore, delaying the digestion of dietary carbohydrates by inhibiting carbohydrase activities reduces postprandial blood sugar levels, exhibiting antidiabetic and anti-obesity properties (Lankatillake et al., 2021).

### 3.4. Chemometric evaluation

The chemometric approach was performed to collectively

analyze and maximize the information obtained from the different chemical characteristics and their relationships. During the fermentation of *A. bunius* fruit juice, the quality of the sample can be characterized by active polyphenols and bioactivities as depicted in the correlation matrix and PCA biplot. Positive correlations between variables during fermentation reveal that the decline in DPPH $\cdot$  is attributed to changes in most phenolic acids, rutin, and quercetin (Fig. 6A). Moreover, the increase in ABTS $^{+}$  is associated with decreasing anthocyanin, increasing gallic acid, and alterations in some flavonoids. The trend in H $_2$ O $_2$  is linked to changes in *p*-coumaric acid, *t*-ferulic acid, and quercetin. At the same time, Fe $^{3+}$  reduction is tied to the increasing levels of gallic acid, total flavonoids, and catechin. The increase in  $\alpha$ -amylase is connected to the decrease in total anthocyanin, affecting *t*-ferulic acid, quercetin, syringic acid, and ellagic acid. Conversely, the decrease in  $\alpha$ -glucosidase is associated with the decline in total anthocyanin, influencing *p*-coumaric acid, quercetin, rutin hydrate, ellagic acid, and syringic acid. Among the specific phenolic compounds, gallic acid and catechin were observed to be the most positively correlated with  $\alpha$ -amylase,  $\alpha$ -glucosidase, and H $_2$ O $_2$  scavenging activity.

Multivariate analysis through principal component analysis (PCA) was done in order to characterize and explain the canonical relationships among the analyzed variables (Zielinski et al., 2018). A PCA biplot was utilized to simplify the representation of high-dimensional data during fermentation (Fig. 6B). The score plot, denoting fermentation times, revealed that the major variation could be explained by *Dim1* and *Dim2*, with variances of 46.4% and 23.2%, respectively. The samples were grouped based on fermentation time (0, 24, 48, and 72 h). The biplot depicts the similarities and differences among these groups of samples in terms of bioactivities and the amount of specific phenolic compounds. Examination of the score plot in conjunction with the loading plot, depicting polyphenols and bioactivities, indicated close interrelatedness at 24 hours of fermentation, particularly with the decline in *p*-coumaric acid influenced by changes in H $_2$ O $_2$ , DPPH $\cdot$ , and  $\alpha$ -glucosidase assays. Additionally, the 72-hour point exhibited increased Fe $^{3+}$  reduction associated with polyphenols such as caffeic acid, catechin, resveratrol, and total flavonoids. Close interrelatedness was observed between unfermented and 48-hour fermentation concerning changes in phenolic acids, flavonoids, and total anthocyanins affected by ABTS $^{+}$  and  $\alpha$ -amylase inhibition.



**Fig. 6.** Pearson's correlation matrix (A) and PCA biplot (B) based on the polyphenolic contents and biological activities of *Antidesma bunius* juice fermented by *Lactiplantibacillus plantarum*. Score plots are grouped based on fermentation times. Loading plots indicate polyphenols and bioactivities. TPC, total phenolic content; GA, gallic acid; CA, caffeic acid; SA, syringic acid; EA, ellagic acid; p-CA, para-coumaric acid; t-FA, trans-ferulic acid; TFC, total flavonoid content; TAC, total anthocyanin content; C, catechin; E, epicatechin; RH, rutin hydrate; M, myricetin; R, resveratrol; Q, quercetin; DPPH, DPPH' scavenging activity; FRAP, ferric reducing power; ABTS, ABTS<sup>++</sup> scavenging activity; H<sub>2</sub>O<sub>2</sub>, hydrogen peroxide scavenging activity; α-amy, α-amylase inhibitory activity; α-gluc, α-glucosidase inhibitory activity.

The outcomes of chemometric analyses helped in identifying the patterns and correlations among the phenolic compounds and biological activities as influenced by lactic acid fermentation with *L. plantarum*. In similar studies, the results of chemometric analysis have been useful in classifying

unfermented and fermented beverages in terms of the evolution of both volatile and non-volatile compounds in the fruit substrates. Models from the analysis were also useful in predicting the antioxidant and other bioactivities based on the correlations (Zielinski et al., 2018). Since the process of

fermentation often enhances the phenolic compound concentrations, which are known to have antioxidant and other biological activities, the results of the chemometric analysis in this study revealed that there were specific compounds in the fermented *A. bunius* juice that contributed the most to the desired bioactivities, optimizing the effect of the fermentation process to the functional benefits of the product (Lizardo et al., 2020).

## 4. Conclusions

Lactic acid fermentation of *A. bunius* fruit juice by *L. plantarum* induced dynamic changes in physicochemical properties, polyphenolic contents, and biological activities. The observed shifts in LAB count, TSS, and subsequent recovery align with literature findings, indicating the impact of juice acidity and phenolics on bacterial growth. Polyphenolic analysis revealed a complex profile shift during fermentation. Specific phenolic acids and flavonoids, such as gallic acid and catechin, increased, suggesting a positive influence on biological activities. Fermentation-induced changes positively affected the biological activities, specifically ferric reducing power and hydrogen peroxide scavenging activity, and the  $\alpha$ -amylase enzyme-inhibition activities, revealing the potential of lactic acid fermentation to enhance the functional properties of *A. bunius* juice. The promising *in vitro* enzyme-inhibition activities against  $\alpha$ -amylase and  $\alpha$ -glucosidase highlight its potential as a natural alternative that can be used in the development of functional foods with anti-diabetic potential. Chemometric evaluations emphasized synergistic interactions between polyphenols, particularly gallic acid, and catechin, and bioactivities such as enzyme inhibition and H<sub>2</sub>O<sub>2</sub> scavenging activity, contributing to the overall health-promoting properties of the fermented juice. Taken together, fermented fruit juice presents a promising method for developing a functional beverage with enhanced health benefits.

### Funding

This research was funded by the University of the Philippines System Emerging Inter-Disciplinary Research Program (OVPAA-EIDR-C09-15).

### Acknowledgements

The authors wish to thank Ms. Erin Therese Kelley for

providing the actual codes for chemometric evaluation and to Mr. Lloyd Earl Flandez for the valuable assistance in the HPLC analysis.

### Conflict of interests

The authors declare no potential conflicts of interest.

### Author contributions

Conceptualization: Lizardo-Agustin RCM. Methodology: Nituda JP, Lizardo-Agustin RCM. Formal analysis: Nituda JP. Validation: Lizardo-Agustin RCM. Writing - original draft: Nituda JP. Writing - review & editing: Lizardo-Agustin RCM.

### Ethics approval

This article does not require IRB/IACUC approval because there are no human and animal participants.

### ORCID

Jerald P. Nituda (First author)

<https://orcid.org/0009-0008-6287-8974>

Rona Camille M. Lizardo-Agustin (First & Corresponding author)

<https://orcid.org/0000-0002-3260-3012>

## References

- American Public Health Association. 9215 Heterotrophic plate count. In: Standard Methods for the Examination of Water and Wastewater, Lipps WC, Baxter TE, Braun-Howland E (Editors), APHA Press, Washington DC, USA, p 1-8 (2018)
- Assaad HI, Hou Y, Zhou L, Carroll RJ, Wu G. Rapid publication-ready MS-Word tables for two-way ANOVA. SpringerPlus, 4, 33 (2015)
- Carbonera AFA, Atienza LM, Estacio MAC, Duque SMM, Lizardo-Agustin RCM, Flandez LEL, Castillo-Israel KAT. Effects of various processing methods on the dietary fiber and antioxidant properties of Bignay (*Antidesma bunius* L. Spreng) fruit. Food Chem Adv, 3, 100561 (2023)
- Chopra AS, Lordan R, Horbańczuk OK, Atanasov AG, Chopra I, Horbańczuk JO, Jozwik A, Huang L, Pitgozliev V, Banach M, Battino M, Arkells N. The current use and evolving landscape of nutraceuticals. Pharmacol Res 175, 106001 (2022)
- Crieta BRA, Sartagoda KJD, Flandez LEL, Morales DB, San Pascual AO, Magdalita PM, Castillo-Israel KAT.

- Morphological, physicochemical and proximate composition of two Philippine bignay (*Antidesma bunius* (L.) Spreng) cultivars at different maturity stages. *Trop Agric*, 99, 307-321 (2022)
- de Llano DG, Gil-Sánchez I, Esteban-Fernández A, Ramos AM, Fernández-Díaz M, Cueva C, Moreno-Arribas MV, Bartolome B. Reciprocal beneficial effects between wine polyphenols and probiotics: An exploratory study. *Eur Food Res Technol*, 243, 531- 538 (2017)
- Di Cagno R, Surico RF, Minervini G, Rizzello CG, Lovino R, Servili M, Tatiechi A, Urbani S, Gobbetti M. Exploitation of sweet cherry (*Prunus avium* L.) puree added of stem infusion through fermentation by selected autochthonous lactic acid bacteria. *Food Microbiol*, 28, 900-909 (2011)
- Durmaz L, Kiziltas H, Karagecili H, Alwasel S, Gulcin İ. Potential antioxidant, anticholinergic, antidiabetic and antiglaucoma activities and molecular docking of spiraeoside as a secondary metabolite of onion (*Allium cepa*). *Saudi Pharm J*, 31, 101760 (2023)
- Fattahi S, Zabihi E, Abedian Z, Pourbagher R, Ardekani AM, Mostafazadeh A, Akhavan-Niaki H. Total phenolic and flavonoid contents of aqueous extract of stinging nettle and *in vitro* antiproliferative effect on hela and BT-474 cell lines. *Int J Mol Cell Med*, 3, 102-107 (2014)
- Fessard A, Bourdon E, Payet B, Remize F. Identification, stress tolerance, and antioxidant activity of lactic acid bacteria isolated from tropically grown fruits and leaves. *Can J Microbiol*, 62, 550-561 (2016)
- Filannino P, Di Cagno R, Gobbetti M. Metabolic and functional paths of lactic acid bacteria in plant foods: Get out of the labyrinth. *Curr Opin Biotechnol*, 49, 64-72 (2018)
- Flandez LEL, Castillo-Israel KAT, Rivadeneira JP, Tuano APP, Hizon-Fradejas AB. Development and validation of an HPLC-DAD method for the simultaneous analysis of phenolic compounds. *Malaysian J Fundam Appl Sci*, 19, 855-864 (2023)
- Fortune Business Insights. The global functional food and beverage market is projected to grow from \$281.14 billion in 2021 to \$529.66 billion in 2028 at a CAGR of 9.5%. Available from: <https://www.fortunebusinessinsights.com/functional-foods-market-102269> Accessed Feb. 4, 2024. (2021)
- Gestupa EJJ, Big Things are on its Way for Bignay. BAR digest, DA-Bureau of agricultural research. Available from: <https://www.bar.gov.ph/media-resources/digest> Accessed Nov. 5, 2023. (2015)
- Giusti MM, Wrolstad RE. Characterization and measurement of anthocyanins by UV-Visible spectroscopy. *Curr Protoc Food Anal Chem*, F1.2.1-F1.2.3 (2001)
- Gonçalves AC, Nunes AR, Flores-Félix JD, Alves G, Silva LR. Cherries and blueberries-based beverages: Functional foods with antidiabetic and immune booster properties. *Molecules*, 27, 3294 (2022)
- González-Palma I, Escalona-Buendía HB, Ponce-Alquicira E, Téllez-Téllez M, Gupta VK, Díaz-Godínez G, Soriano-Santos J. Evaluation of the antioxidant activity of aqueous and methanol extracts of *pleurotus ostreatus* in different growth stages. *Front Microbiol*, 7, 1099 (2016)
- Hardinasinta G, Mursalim M, Muhidong J, Salengke S. Determination of some chemical compounds of bignay (*Antidesma bunius*) fruit juice. *Food Sci Technol (Brazil)*, 41, 974-979 (2021)
- Isas AS, Escobar F, Álvarez-Villamil E, Molina V, Mateos R, Lizarraga E, Mozzi F, Nieuwenhove CV. Fermentation of pomegranate juice by lactic acid bacteria and its biological effect on mice fed a high-fat diet. *Food Biosci*, 53, 102516 (2023)
- Islam S, Ahammed S, Sukorno FI, Koly SF, Biswas M, Hossain S. A review on phytochemical and pharmacological potentials of *Antidesma bunius*. *J Anal Pharm Res*, 7, 602-604 (2018)
- Jamar G, Estadella D, Pisani LP. Contribution of anthocyanin-rich foods in obesity control through gut microbiota interactions. *BioFactors*, 43, 507-516 (2017)
- Kazeem MI, Adamson JO, Ogunwande IA. Modes of inhibition of  $\alpha$ -amylase and  $\alpha$ -glucosidase by aqueous extract of morinda lucida benth leaf. *Biomed Res Int*, 2013, 527570 (2013)
- Lankatillake C, Luo S, Flavel M, Lenon GB, Gill H, Huynh T, Dias DA. Screening natural product extracts for potential enzyme inhibitors: Protocols, and the standardisation of the usage of blanks in  $\alpha$ -amylase,  $\alpha$ -glucosidase and lipase assays. *Plant Methods*, 17, 1-19 (2021)
- Li F, Ding Z, Ke W, Xu D, Zhang P, Bai J, Mudassar S, Muhammad I, Guo X. Ferulic acid esterase-producing lactic acid bacteria and cellulase pretreatments of corn stalk silage at two different temperatures: Ensiling characteristics, carbohydrates composition and enzymatic saccharification. *Bioresour Technol*, 282, 211-221 (2019)
- Liao W, Shen J, Manickam S, Li S, Tao Y, Li D, Liu D, Han Y. Investigation of blueberry juice fermentation by mixed probiotic strains: Regression modeling, machine learning optimization and comparison with fermentation by single strain in the phenolic and volatile profiles. *Food Chem*, 405, 134982 (2023)
- Lizardo RCM, Cho HD, Won YS, Seo KI. Fermentation with mono- and mixed cultures of *Lactobacillus plantarum* and *L. casei* enhances the phytochemical content and biological activities of cherry silverberry (*Elaeagnus multiflora* Thunb.) fruit. *J Sci Food Agric*, 100, 3687-3696 (2020)

- Lordan R. Dietary supplements and nutraceuticals market growth during the coronavirus pandemic: Implications for consumers and regulatory oversight. *Pharma Nutr*, 18, 100282 (2021)
- Mousavi ZE, Mousavi SM, Razavi SH, Emam-Djomeh Z, Kiani H. Fermentation of pomegranate juice by probiotic lactic acid bacteria. *World J Microbiol Biotechnol*, 27, 123-128 (2011)
- Mustafa SM, Chua LS, El-Enshasy HA. Effects of agitation speed and kinetic studies on probiotication of pomegranate juice with *Lactobacillus casei*. *Molecules*, 24, 2357 (2019)
- Nualkaekul S, Lenton D, Cook MT, Khutoryanskiy VV, Charalampopoulos D. Chitosan coated alginate beads for the survival of microencapsulated *Lactobacillus plantarum* in pomegranate juice. *Carbohydr Polym*, 90, 1281-1287 (2012)
- Palencia-Argel M, Rodríguez-Villamil H, Bernal-Castro C, Díaz-Moreno C, Fuenmayor CA. Probiotics in anthocyanin-rich fruit beverages: Research and development for novel synbiotic products. *Crit Rev Food Sci Nutr*, 64, 110-126 (2022)
- Plessas S. Advancements in the use of fermented fruit juices by lactic acid bacteria as functional foods: Prospects and challenges of *Lactiplantibacillus (Lpb.) plantarum* subsp. *plantarum* application. *Fermentation*, 8, 6 (2022)
- Razola-Díaz M del C, De Montijo-Prieto S, Aznar-Ramos MJ, Jiménez-Valera M, Ruiz-Bravo A, Verardo V, Gomez-Caravaca AM. Effect of lactic acid bacteria fermentation on the polar compounds content with antioxidant and antidiabetic activity of avocado seed extracts. *Fermentation*, 9, 420 (2023)
- Recuenco MC, Lacsamana MS, Hurtada WA, Sabulase VC. Total phenolic and total flavonoid contents of selected fruits in the Philippines. *Philipp J Sci*, 145, 275-281 (2016)
- Rozès N, Peres C. Effects of phenolic compounds on the growth and the fatty acid composition of *Lactobacillus plantarum*. *Appl Microbiol Biotechnol*, 49, 108-111 (1998)
- Ruiz Rodríguez LG, Zamora Gasga VM, Pescuma M, Van Nieuwenhove C, Mozzi F, Sánchez Burgos JA. Fruits and fruit by-products as sources of bioactive compounds. Benefits and trends of lactic acid fermentation in the development of novel fruit-based functional beverages. *Food Res Int*, 140, 109854 (2021)
- Sartagoda KJ, Ilano MC, Flandez LE, Castillo-Israel KA. Evaluation of the antioxidant activity of bignay (*Antidesma bunius* (Linn.) Spreng var. Kalabaw) flesh and seeds as affected by maturity and processing method. *CMUJ Nat Sci*, 20, e2021042 (2021)
- Septembre-Malaterre A, Remize F, Pouchet P. Fruits and vegetables, as a source of nutritional compounds and phytochemicals: Changes in bioactive compounds during lactic fermentation. *Food Res Int*, 104, 86-99 (2018)
- Sheehan VM, Ross P, Fitzgerald GF. Assessing the acid tolerance and the technological robustness of probiotic cultures for fortification in fruit juices. *Innovative Food Sci Emerging Technol*, 8, 279-284 (2007)
- Sultana K, Jayathilakan K, Pandey MC. Evaluation of antioxidant activity, radical scavenging, and reducing power of clove oil and clove oleoresin in comparison with natural and synthetic antioxidants in chevon (*Capra aegagrus hircus*) and chicken meat. *Def Life Sci J*, 3, 51-58 (2017)
- Sun Y, Cao Q, Huang Y, Lu T, Ma H, Chen X. Mechanistic study on the inhibition of  $\alpha$ -amylase and  $\alpha$ -glucosidase using the extract of ultrasound-treated coffee leaves. *J Sci Food Agric*, 104, 63-74 (2024)
- Szutowska J. Functional properties of lactic acid bacteria in fermented fruit and vegetable juices: A systematic literature review. *Eur Food Res Technol*, 246, 357-372 (2020)
- Thbayh DK, Fiser B. Computational study of synthetic and natural polymer additives: Antioxidant potential of BHA, TBHQ, BHT, and curcumin. *Polym Degrad Stab*, 201, 109979 (2022)
- Tian L, Tan Y, Chen G, Wang G, Sun J, Ou S, Chen W, Bai W. Metabolism of anthocyanins and consequent effects on the gut microbiota. *Crit Rev Food Sci Nutr*, 59, 982-991 (2019)
- Tsoupras A, Zabetakis I, Lordan R. Functional foods: Growth, evolution, legislation, and future perspectives. In: *Functional Foods and Their Implications for Health Promotion*. Elsevier Inc., New York, USA, p 367-377 (2023)
- Tyl C, Sadler GD. pH and titratable acidity. In: *Food Analysis*, 5th ed, Nielsen SS (Editor), Springer, New York, USA, p 389-406 (2017)
- Udomkasemsab A, Ngamlerst C, Kwanbunjun K, Krasae T, Amnuaysookkasem K, Chunthanom P, Prangthip P. Maoberry (*Antidesma bunius*) improves glucose metabolism, triglyceride levels, and splenic lesions in high-fat diet-induced hypercholesterolemic rats. *J Med Food*, 22, 29-37 (2019)
- Vivek k, Mishra S, Pradhan RC, Jayabalan R. Effect of probiotification with *Lactobacillus plantarum* MCC 2974 on quality of Sohiong juice. *LWT - Food Sci Technol*, 108, 55-60 (2019)
- Wang Y, Li H, Ren Y, Wang Y, Ren Y, Wang X, Yue T, Wang Z, Gao Z. Preparation, model construction and efficacy lipid-lowering evaluation of kiwifruit juice fermented by probiotics. *Food Biosci*, 47, 101710 (2022)

- Wootton-Beard PC, Ryan L. Improving public health?: The role of antioxidant-rich fruit and vegetable beverages. *Food Res Int*, 44, 3135-3148 (2011)
- Wu M, Yang Q, Wu Y, Ouyang J. Inhibitory effects of acorn (*Quercus variabilis* Blume) kernel-derived polyphenols on the activities of  $\alpha$ -amylase,  $\alpha$ -glucosidase, and dipeptidyl peptidase IV. *Food Biosci*, 43, 101224 (2021)
- Yang J, Ji Y, Park H, Lee J, Park S, Yeo S, Shin H, Holzapfel WH. Selection of functional lactic acid bacteria as starter cultures for the fermentation of Korean leek (*Allium tuberosum* Rottler ex Sprengel.). *Int J Food Microbiol*, 191, 164- 171 (2014)
- Youn JS, Kim YJ, Na HJ, Jung HR, Song CK, Kang SY, Kim JY. Antioxidant activity and contents of leaf extracts obtained from *Dendropanax morbifera* LEV are dependent on the collecting season and extraction conditions. *Food Sci Biotechnol*, 28, 201-207 (2019)
- Zhao YS, Eweys AS, Zhang JY, Zhu Y, Bai J, Darwesh OM, Zhang HB, Xiao X. Fermentation affects the antioxidant activity of plant-based food material through the release and production of bioactive components. *Antioxidants*, 10, 2004 (2021)
- Zhu Y, Sun H, He S, Lou Q, Yu M, Tang M, Tu L. Metabolism and prebiotics activity of anthocyanins from black rice (*Oryza sativa* L.) *in vitro*. *PLoS One*, 13, e0195754 (2018)
- Zielinski AA, Alberti A, Nogueira A. Characterizing fruit juices and fermented fruit beverages using chemometrics tools. In: *Fruit Juices*, Academic Press, Cambridge, MA, USA, p 823-833 (2018)
- Zubia CS, Babaran GMO, Duque SMM, Mopera LE, Flandez LEL, Castillo-Israel KAT, Reginio Jr FC. Impact of drying on the bioactive compounds and antioxidant properties of bignay (*Antidesma bunius* (L.) Spreng.) pomace. *Food Prod Process Nutr*, 5, 1-13 (2023)