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Research Article

Comparative Evaluation of the Antioxidant Capacities, Organic Acids, and Volatiles of Papaya Juices Fermented by *Lactobacillus acidophilus* and *Lactobacillus plantarum*

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Fermentation of foods by lactic acid bacteria is a useful way to improve the nutritional value of foods. In this study, the health-promoting effects of fermented papaya juices by two species, *Lactobacillus acidophilus* and *Lactobacillus plantarum*, were determined. Changes in pH, reducing sugar, organic acids, and volatile compounds were determined, and the vitamin C, total phenolic content, and flavonoid and antioxidant capacities during the fermentation process were investigated. Juices fermented by *Lactobacillus acidophilus* and *Lactobacillus plantarum* had similar changes in pH and reducing sugar content during the 48 h fermentation period. Large amounts of aroma-associated compounds and organic acids were produced, especially lactic acid, which increased significantly ($p < 0.05$) (543.18 mg/100 mL and 571.29 mg/100 mL, resp.), improving the quality of the beverage. In contrast, the production of four antioxidant capacities in the fermented papaya juices showed different trends after 48 hours' fermentation by two bacteria. *Lactobacillus plantarum* generated better antioxidant activities compared to *Lactobacillus acidophilus* after 48 h of fermentation. These results indicate that fermentation of papaya juice can improve its utilization and nutritional effect.

1. Introduction

Lactic acid fermentation is one of the oldest and most economical methods used in food preservation, especially as a "natural" process that enhances the efficacy and quality of foods while improving the organoleptic qualities of the product [1–3]. In fact, at the end of the twentieth century, the Food and Agriculture Organization (FAO) of the United Nations recognized the importance of fermented products, highlighting their economic importance for local communities in developing countries [4]. Probiotic foods are an important and dominating part of the functional food market, accounting for 60% to 70% of the total functional food market [5], with more than 370 products launched worldwide in Japan and Europe in 2005 [6]. A number of studies have reported that the development of fruit juice-based fermented beverages would be the next food category in which health-promoting probiotic bacteria will make their mark [7, 8]. Many countries have conducted extensive research and development on probiotic-fermented fruit and vegetable beverages in recent years, especially in Korea, India, and Brazil. These studies have used watermelons, tomatoes, apples, and so on as raw materials for fermentation by lactic acid bacteria [9–11]. An important aspect of lactic acid bacteria (LAB) fermentation is the production of organic acids, sugar polymers, aromatic compounds, vitamins, polyphenolic compounds, and some useful enzymes, which enrich the human diet [12, 13]. The fermentation products generated by different lactic acid bacteria are not the same. Lee et al. [14] showed variations among the respective LAB strains from kimchi, and Kumar et al. [15] showed that *L. plantarum Lp91* has a better effect than *Lp21* in the management of hypercholesterolaemia. Thus, significant differences in the antioxidant activity and composition of fermented products can be generated by specific probiotic strains [16].

Papaya (*Carica papaya* L.), a member of the Caricaceae family, is widely cultivated in tropical and subtropical regions and is marketed around the world for its great taste and nutrients [17, 18]. Papaya has been identified as a valuable source of nutrients and antioxidants and is also used for traditional

medicine. Its fruits, stems, leaves, and roots are used in a wide range of medical applications, including the production of two important bioactive compounds (chymopapain and papain), which are widely used for digestive diseases [19–21]. However, postharvest losses of papaya occur throughout the value chain due to rapid deterioration of its chemical components and pulp softening, which results in a short shelf life of the fresh fruit [22, 23]. Many studies have demonstrated the feasibility of using fruits, such as oranges, pineapples, bananas, and cranberries, to produce probiotic beverages [24]. However, other fruits, such as papaya, have not been adequately studied. Thus far, only a small amount of research has been conducted on fermented papaya beverages. Among these, most have studied the use of papaya for wine-making [25, 26]. Di Cagno et al. [27] demonstrated the growth and survival of *L. plantarum* and *L. pentosus* in a papaya juice-based medium. All of these studies have demonstrated that papaya beverages are suitable for microbial growth. To explore the feasibility of LAB fermentation of papaya beverages to improve the utilization value of papaya, this study assessed two lactic acid bacteria for fermentation.

2. Materials and Methods

2.1. Materials

Samples and Bacteria. Papaya puree, skim milk powder, and edible glucose were purchased from the Nanguo Supermarket and Hainan Dachuan Food Co., Ltd. (Haikou, China). *Lactobacillus acidophilus GIM1.731 (L. acidophilus)* and *Lactobacillus plantarum GIM1.140 (L. plantarum)* were purchased from Guangdong Microbiology Culture Center (Guangzhou, China).

Preparation of Papaya Juices and Fermentation. Each sample was formulated to 200 g for fermentation. According to mass, 45% papaya puree, 45% distilled water, 5% edible glucose, and 5% skim milk powder (10-fold diluted skim milk powder) were added to a conical flask, heat sterilized at 90°C for 10 min, and then cooled in a water bath. Each LAB strain was inoculated at 5% of the mass ratio of the fermentation broth, and the mixture was incubated at 37°C for 48 h under static conditions. Samples were stored at −80°C. Fermentation processes were repeated three times.

2.2. Methods

2.2.1. Determination of pH. The pH of the samples was measured by a digital pH meter (FE20 laboratory pH meter).

2.2.2. Determination of Reducing Sugars. The reducing sugars content of fermented juice was analyzed as glucose equivalents by the 3,5-dinitrosalicylic acid (DNS) method of Saqib [28] with some modification. In this application, 2 mL of 250-fold diluted sample and 4 mL DNS reagent were added to the 10-mL graduated tube and heated in a boiling water bath for 5 minutes. When removed, the tube was immediately placed in cold water, cooled to room temperature, supplemented with water to 10 mL, and shaken well. The absorbance was measured at 540 nm. The results were expressed as milligrams of glucose equivalents.

2.2.3. Determination of Vitamin C. The 2,6-dichlorophenolindophenol titrimetric method was used to measure the levels of reduced ascorbic acid [29].

2.2.4. Determination of Total Phenolic Content. Extracts were prepared by mixing 5 g of sample with 25 mL of 50% ethanol. The mixture was placed in a thermostat oscillator set at 100 r/min for 1 h and was then centrifuged at 4000 r/min for 10 min. Extracts were transferred into test conical flasks and stored at 4°C before analysis. Total phenolic content was determined using the Folin-Ciocalteu method described by de Sà et al. [30] with some modifications. A 1 mL aliquot of each fermented papaya beverage extract was mixed with 0.2 mL of Folin-Ciocalteu's reagent and was incubated at room temperature for 3 min. Then, the mixture was dissolved in 7.5% Na_2CO_3 and adjusted to a volume of 10 mL. After 30 min, the absorbance was measured at 765 nm. The results were expressed as milligrams of gallic acid equivalents (GAE).

2.2.5. Determination of Total Flavonoids Content. The total flavonoids content of samples was measured according to the method of Wu et al. [31] with some modifications. A 0.6 mL aliquot of each sample was mixed with 1.2 mL of 80% methanol; then 0.18 mL of 20% $NaNO_2$ was added. After 6 min, 0.36 mL of 8% $Al(NO_3)_3$ was added, followed by the addition of 1.2 mL of 1 mol/L NaOH after 5 min. After 15 min, the absorbance was measured at 510 nm. The results were expressed as milligrams of rutin equivalents (RE).

2.2.6. Determination of Total Carotenoid Content. The total carotenoid content was measured according to the method of Carbonell-Capella et al. [32] with some modification. A 1 mL aliquot of each sample was homogenized with 5 mL of extraction solvent (hexane/acetone/methanol, 50 : 25 : 25, *v*/*v*). The mixture was placed in a thermostat oscillator set at 100 r/min for 30 min and then was centrifuged at 6000 r/min at 4°C for 10 min. The top, colored hexane layer was recovered and transferred to a 25 mL volumetric flask, and the volume was then adjusted to 25 mL with hexane. The absorbance of the samples was measured at 450 nm. The results were calculated as described by Ritter and Purcell (1981) using the extinction coefficient of β-carotene, $E^{1\%} = 2505$.

2.2.7. Determination of the Total Antioxidant Capacity In Vitro

(1) 2,2-Diphenyl-1-Picrylhydrazyl (DPPH) Radical Scavenging Assay. The $DPPH^{\bullet+}$ assay was performed according to Li et al. [33] with some modifications. Briefly, 0.02 g of DPPH was transferred to a 25 mL volumetric flask, the volume was adjusted to 25 mL with methanol, and, after the flask was fully oscillated, the solution was subjected to ultrasound for 5 min. The 0.8 mg/mL solution of DPPH in methanol was diluted with methanol until the solution had an absorbance of 1.2–1.3 at 517 nm. A 0.12 mL aliquot of sample was added to 4 mL of the DPPH radical solution. The mixture was incubated in the

dark for 45 min, after which the absorbance was measured at 517 nm. The DPPH radical scavenging activity (% inhibition) was calculated using the formula $(1 - A_1/A_0) \times 100$, where A_0 is the absorbance of the reagent blank and A_1 is the absorbance of the sample.

(2) 2,2′-Azinobis-(3-Ethylbenzothiazoline-6-Sulfonate) (ABTS) Radical Scavenging Assay. The $ABTS^{\bullet+}$ assay was performed according to Um et al. [34] with some modification. First, 0.192 g of ABTS and 0.067 g of $K_2S_2O_8$ were dissolved in phosphate buffered saline (PBS, pH = 7), transferred to a 50 mL volumetric flask and the volume was adjusted to 50 mL. The working solution of $ABTS^{+\bullet}$ was obtained by diluting the stock solution in PBS to give an absorption of 0.70 ± 0.02 at 734 nm. A 50 μL aliquot of each sample was added to 4 mL of the $ABTS^{+\bullet}$ solution and absorbance readings at 734 nm were taken at every 10 min.

The ABTS radical scavenging activity (% inhibition) was calculated using the formula $(1 - A_1/A_0) \times 100$, where A_0 is the absorbance of the reagent blank and A_1 is the absorbance of the sample.

(3) Ferric Reducing Antioxidant Power (FRAP) Assay. The antioxidant capacity was determined using the FRAP assay, which was performed according to Morales-Soto et al. [35] with some modification. The freshly prepared FRAP solution contained 25 mL of 0.3 mol/L acetate buffer (pH 3.6), 2.5 mL of 10 mmol/L 2,4,6-tripyridyl-1,3,5-triazine (TPTZ) (dissolved in 40 mmol/L HCl), and 2.5 mL of 20 mmol/L ferric chloride. A 0.2 mL aliquot of each 10-fold diluted sample was mixed with 4 mL of FRAP solution and was incubated for 50 min at room temperature. The ferric reducing ability was measured by monitoring the absorbance at 593 nm using a spectrophotometer (TU-1810, Puxi, Beijing, China) and the FRAP solution was used as a blank. $FeSO_4$ was used as a control to obtain the standard curve. The FRAP values were calculated relative to the activity of $FeSO_4$ and expressed as $FeSO_4$ equivalents.

(4) Cupric Ion Reducing Antioxidant Capacity (CUPRAC) Assay. The typical CUPRAC method, as described by Kondakçi et al. [36], was performed as follows. A solution comprised of 1 mL of 5 mmol/L copper sulfate, 1 mL of 3.75 mmol/L neocuproine (dissolved in methanol), and 1 mL of ammonium acetate buffer at pH 7.0 was prepared. Then, 0.1 mL of sample solution and 0.9 mL of distilled water were added and well mixed. This final mixture was incubated at room temperature for 30 min in a stoppered test tube, after which the absorbance at 450 nm was measured against a reagent blank. Trolox solution was used as a control to obtain the standard curve. The CUPRAC value was calculated relative to the activity of trolox and was expressed as trolox equivalents.

2.2.8. Liquid Chromatography Analysis of Organic Acids. To avoid affecting the determination of the organic acid content, the supernatant was obtained by centrifugation before the measurement to remove the protein [37]. In brief, 2 mL of sample was added to 2 mL of a solution in 1.8% $Ba(OH)_2$, adding 2 mL of a solution of 2% $ZnSO_4$. The mixture was vortex shaken, allowed to settle for 15 min, and centrifuged at 8000 r/min for 10 min. Measurements of organic acid contents were performed according to the method described by Zhao et al. [38]. The sample was filtered through a 0.22 μm pore size membrane filter before injection. An HPLC system (Agilent 1260, Agilent Technologies Inc., USA) equipped with a pump system and a UV-visible detector was used to monitor the absorbance at 210 nm for the analysis of organic acids. Organic acids were simultaneously analyzed on a ZORBAX SB-Aq column (250 mm × 4.6 mm, 5 μm) (Agilent, Agilent Technologies Inc., USA), which was kept at 30°C. The assay conditions used were as follows: the flow was set at 0.8 mL/min and the eluent consisted of 0.02 mol/L ammonium dihydrogen phosphate (pH 2.66, adjust pH by phosphate) with 3% methanol. The flow ratio was isocratic elution of Solvent A (ammonium dihydrogen phosphate) : Solvent B (methanol) (97 : 3). The chromatographic peaks corresponding to each organic acid were identified by comparing the retention times with those of standards. For each compound, a calibration curve was prepared using standards to determine the relationship between the peak area and organic acid concentration.

2.2.9. Analysis of Volatile Compounds. The volatile compound analysis was performed according to the method described by Fuggate et al. [39] with some modification. Volatile components in the headspace were trapped by solid phase microextraction (SPME). The dioctanol was used as an internal standard. For each sample, a 1 mL aliquot was taken from a 20 mL sealed headspace bottle. A CTC Trinity Autosampler was used for all assays, with a SPME fibre of 50/30 μm DVB/CAR/PDMS. The following extraction conditions were used: temperature, 50°C; equilibration of the fibre, 15 min; extraction time, 30 min; agitation speed, 250 r/min; injection time, 4 min.

A USA Agilent 7890A-5975C Gas Chromatography-Mass Spectrometer (GC-MS) equipped with a DB-wax (30 m long × 0.25 mmID × 0.25 μm df) column was used for all analyses. The chromatographic conditions included a flow rate of 1 mL/min using helium (99.999%) as a carrier gas and an injection temperature of 260°C. The column temperature was maintained at 40°C for 5 min, was ramped at 5°C/min to 250°C, and then was held for 5 min. The power supply mode was set to electronic ionization (EI). The interface, ion source, and quadrupole temperatures were 260, 230, and 150°C, respectively; the electron energy used was 70 eV, and the detector voltage was 2235 V; the scan mode was set at full scan with a mass range of 20–400 amu, using the NIST 2011 library.

2.2.10. Statistical Analysis. The statistical analysis was conducted with DPS ver. 15.10 software and SPSS ver. 17.0 software (Chicago, IL, USA). Results were recorded as the means ± standard deviation (SD) of triplicate experiments. Analysis of variance (ANOVA) was performed on data sets, and significant differences ($p < 0.01$ and $p < 0.05$) between the means were determined by Duncan's multiple range test.

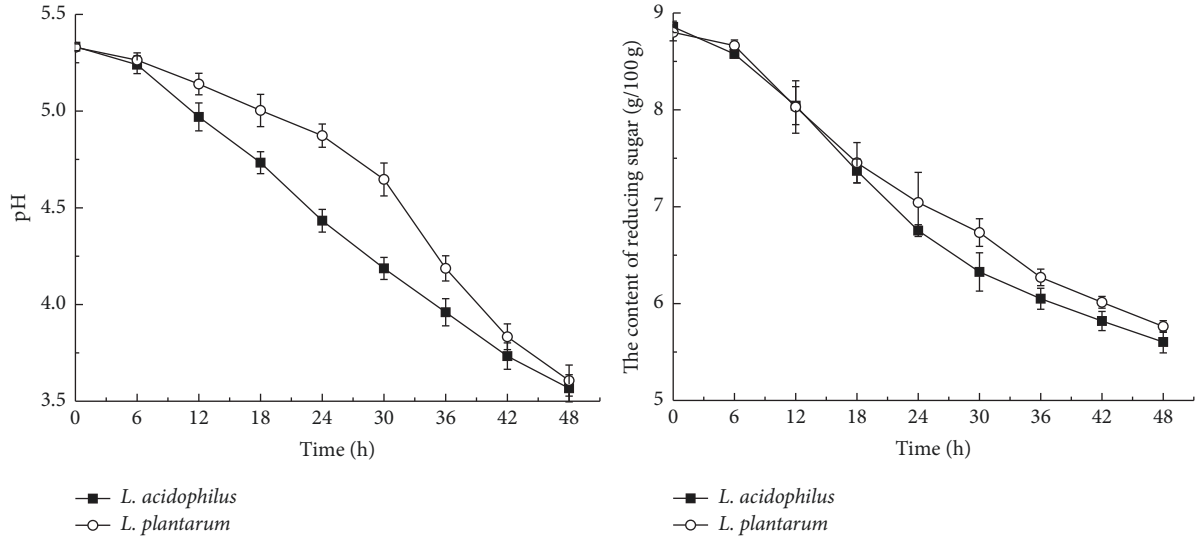

Figure 1: Changes in pH and reducing sugar content of the fermented papaya juices.

3. Results and Discussion

3.1. Fermentation Characteristics of the Papaya Juice during the 48 h Fermentation. The pH value is an important parameter to gauge the progress and end point of lactic acid fermentation, influencing the flavor of the fermented product [40]. The two cultures showed similar characteristics with respect to changes in pH values and in the content of reducing sugars. The changes in pH values of the two probiotic cultures are shown in Figure 1, with the pH of both cultures decreasing significantly during the fermentation process ($p < 0.05$). For example, after 48 h fermentation the pH of fermented papaya juice with *L. acidophilus* (FPJA) decreased from 5.36 to 3.60, and the pH of fermented papaya juice with *L. plantarum* (FPJP) decreased from 5.34 to 3.55. Klupsaite et al. [41] reported that the fermentation of narrow-leaved lupine resulted in similar changes in pH values. Changes in reducing sugar content of the fermented papaya juices are shown in Figure 1, and the content of reducing sugar of both FPJA and FPJP decreased significantly during fermentation ($p < 0.05$). The reducing sugar content of FPJA decreased from 8.86 g/100 g to 5.60 g/100 g, and the reducing sugar content of FPJP decreased from 8.80 g/100 g to 5.76 g/100 g. However, the fermented papaya juices still had a high content of reducing sugar, retaining enough sweetness.

3.2. The Relationship between Antioxidative Components and Antioxidant Capacities

3.2.1. Antioxidative Components of the Fermented Papaya Juices after the 48 h Fermentation Period. A large number of studies analyzing the bioactive composition of papaya have been reported. Papaya is rich in total phenols, carotenoids, flavonoids, vitamin C, and other bioactive substances [42]. The total phenolic content of FPJA decreased more significantly than that of FPJP, as shown in Table 1 ($p < 0.05$). The total phenolic content of FPJA decreased from 31.89 mg/100 g to 27.16 mg/100 g, a decrease of 14.83%, while the total phenolic content of FPJP decreased from 32.09 mg/100 g to 29.53 mg/100 g, representing a decrease of 7.98%. The total flavonoids content of both FPJA and FPJP increased significantly after fermentation as shown in Table 2 ($p < 0.05$). The total flavonoids content of FPJA increased from 0.50 mg/100 g to 1.11 mg/100 g, and the total flavonoids content of FPJP increased to 1.45 mg/100 g. The total carotenoid content of FPJA decreased from 15.47 mg/100 g to 11.48 mg/100 g, and vitamin C content decreased from 21.11 mg/100 g to 17.56 mg/100 g. The total carotenoid content of FPJP decreased from 15.47 mg/100 g to 11.15 mg/100 g, and the vitamin C content decreased from 21.11 mg/100 g to 18.22 mg/100 g.

Table 1: Changes in total phenolic content of the fermented papaya juices.

Fermentation time (h)	Total phenolic content of the fermented papaya juices (mg/100 g)	
	L. acidophilus	*L. plantarum*
0	31.9 ± 0.6^{a}	32.1 ± 0.8^{ab}
6	28.8 ± 0.5^{cd}	31.9 ± 0.4^{ab}
12	30.2 ± 0.3^{bc}	29.8 ± 0.4^{c}
18	30.7 ± 0.7^{ab}	32.5 ± 1.8^{a}
24	31.1 ± 0.8^{ab}	30.7 ± 0.8^{bc}
30	32.1 ± 0.8^{a}	33.0 ± 1.5^{a}
36	29.1 ± 1.97^{c}	31.7 ± 1.6^{ab}
42	27.4 ± 0.4^{de}	30.5 ± 0.5^{bc}
48	27.2 ± 0.9^{e}	29.5 ± 0.6^{c}

Values are expressed as the mean ± SD. Values with different letters (a ~ e) in the same column are significantly different at $p < 0.05$.

Table 2: Changes in others antioxidative components of the fermented papaya juices.

Antioxidative components	Papaya juice (mg/100 mL)	*L. acidophilus* 48 h (mg/100 mL)	*L. plantarum* 48 h (mg/100 mL)
Total flavonoids content	0.50 ± 0.06^{c}	1.11 ± 0.17^{b}	1.45 ± 0.13^{a}
Total carotenoid content	15.5 ± 1.3^{a}	11.5 ± 1.3^{b}	11.1 ± 1.0^{b}
Vitamin C content	21.1 ± 1.0^{a}	17.6 ± 1.0^{b}	18.22 ± 1.0^{b}

Values are expressed as the mean ± SD. Values with different letters (a ~ c) in the same column are significantly different at $p < 0.05$.

3.2.2. Antioxidative Activity of the Fermented Papaya Juices after the 48 h Fermentation Period. Different antioxidant compounds may act against oxidizing agents through distinct mechanisms so that a single isolation method cannot completely evaluate the antioxidant capacity of samples [43]. For this reason, four methods of assessing antioxidants were used to study the antioxidant capacity of FPJ (Table 1).

The antioxidant activity of FPJ determined by the DDPH radical scavenging assay is shown in Table 3. The DPPH radical scavenging activities of FPJA decreased significantly after the fermentation process ($p < 0.05$), and the inhibition decreased from 81.90% to 55.60%. The DPPH radical scavenging activity of FPJP ranged from 77.39% to 86.25%, and the inhibition increased by 4.63%. A similar change in the DPPH radical scavenging has been reported in other studies [44, 45]. The inhibition of FPJ showed that for both cultures there was >50% of radical scavenging activity after 48 h of fermentation.

The results of the ABTS radical scavenging assays are shown in Table 3. The ABTS radical scavenging activity of FPJA decreased significantly after 48 h fermentation ($p < 0.05$). In contrast, the ABTS radical scavenging activity of FPJP tended to increase after fermentation, although no significant difference was observed. In both samples, the inhibition remained >80% after the fermentation. Kim et al. [46] reported that fermented potato juices with *Lactobacillus casei* have a similar change in ABTS radical scavenging activity.

Similarly, the FRAP and CUPRAC values in FPJA decreased significantly after the 48 h fermentation period ($p < 0.05$) (Table 3). Hence, FRAP decreased by 20.60% (decrease from 5.68 to 4.51 mM $FeSO_4$) and CUPRAC decreased by 6.45% (decreasing from 1.24 to 1.16 mM trolox). The FRAP and CUPRAC values of FPJP increased slightly after the 48 h fermentation period ($p < 0.05$) (Table 3). Hence, FRAP increased from 5.54 to 5.74 mM $FeSO_4$ (n.s.), and CUPRAC increased from 1.26 to 1.57 mM trolox ($p < 0.05$).

Both strains have the same pattern for the content of antioxidant compounds. We observed that the total phenolic content of both strains had maximal values at 30 hours and then decreased (Table 1). The reasons for the decrease in the phenolic compounds in the papaya juices during probiotics fermentation likely include their precipitation or oxidation during the process, the combination or adsorption of phenolic compounds with solids, proteins, or even yeasts, and polymerization, all of which results in important losses of these compounds [47]. In contrast, the total flavonoids content of both strains increased. This could be explained by enzymatic degradation and by acids produced by the strain facilitating the release of phenolics and flavanones from their complexed forms in dietary fibre into freely soluble forms by the fermenting microorganism [12, 48]. Similar results were observed in the study of Kantachote et al. [49] who fermented coconut water with *Lactobacillus plantarum DW12*. There was a significant decrease in the total carotenoid content and vitamin C of papaya juices after fermentation by the two cultures ($p < 0.05$). The reason for the decreased total carotenoid content and vitamin C is that carotenoids and vitamin C are easily oxidized at high fermentation temperatures [50, 51].

In recent years, many scholars have become concerned about the antioxidant activity of fermented foods. Ohata et al. [52] studied fermented meat sauce, observing an increase in the DPPH radical scavenging activity. Simsek et al. [53] studied fermented vegetable juices but found no significant difference between the DPPH radical scavenging activity of fermented and unfermented vegetable juices. Nazzaro et al. [54] showed a decrease in the DPPH radical scavenging activity of fermented carrot juices with *L. rhamnosus* and also showed an increase in the DPPH radical scavenging activity of fermented carrot juices with *L. bulgaricus* after 48 h. In addition, Gan et al. [55] reported that there is no change in the ABTS free radical scavenging capacity of fermented mung bean with *L. plantarum WCFS1*.

Overall, the two probiotic cultures showed different trends of antioxidant capacities. Li et al. [56] studied the effect of onion juice on the fermentation of milk by L. acidophilus, demonstrating that the antioxidant activity decreased significantly during the fermentation process, confirming that it was necessary to scavenge radicals for *L. acidophilus* growth. Hervert-Hernández et al. [57] studied the stimulatory role of grape pomace polyphenols on *L. acidophilus* growth and inferred that *L. acidophilus* may be able to use polyphenols possessing antioxidant functions as substrates. Our results are supported by the research of Ankolekar et al. [58], who observed a decrease in total phenolic content and antioxidant capacities throughout fermentation by *L. acidophilus*. Das and Goyal [59] reported that *L. plantarum* shows better antioxidant activity compared to *L. acidophilus* and can act as an antioxidative probiotic. *L. plantarum* fermentation broth has a strong reducing ability, Fe^{2+} chelating ability, and a variety of free radical scavenging abilities, as demonstrated by the work of Tang et al. [60]. All of these studies help to further illustrate our experimental results.

3.3. The Organic Acid Contents of the Fermented Papaya Juices. Lactic acid bacteria catabolize sugars via fermentation, leading to the formation of organic acids (including lactic acid, acetic acid), with ethanol as the final products [61, 62]. *L. acidophilus* is an obligately homofermentative bacteria (produces lactic acid as main metabolic product) while *L. plantarum* belongs to the facultatively homofermentative

Table 3: Change in antioxidant activity of the fermented papaya juices.

Fermentation time (h)	DPPH (% Inh[A])		ABTS (% Inh)	
	L. acidophilus	*L. plantarum*	*L. acidophilus*	*L. plantarum*
0	81.9 ± 1.2^{a}	79.5 ± 0.7^{c}	90.5 ± 2.0^{a}	88.5 ± 1.0^{a}
6	77.1 ± 2.9^{a}	77.4 ± 0.4^{d}	86.8 ± 3.1^{bc}	84.9 ± 0.9^{d}
12	78.8 ± 0.4^{a}	78.5 ± 0.4^{cd}	86.9 ± 2.6^{bc}	86.2 ± 0.4^{c}
18	78.0 ± 0.4^{a}	81.4 ± 0.5^{b}	87.5 ± 0.8^{ab}	87.6 ± 0.6^{abc}
24	70.6 ± 0.1^{b}	83.0 ± 1.2^{b}	88.1 ± 0.7^{ab}	86.9 ± 1.1^{bc}
30	66.9 ± 3.96^{b}	86.3 ± 1.1^{a}	89.8 ± 0.9^{ab}	87.6 ± 0.7^{ab}
36	77.7 ± 2.2^{a}	82.7 ± 0.9^{b}	90.6 ± 1.3^{a}	87.9 ± 0.6^{ab}
42	60.8 ± 5.7^{c}	83.5 ± 1.7^{b}	86.9 ± 0.7^{c}	88.8 ± 0.7^{a}
48	55.6 ± 1.5^{d}	83.1 ± 1.9^{b}	84.2 ± 1.0^{c}	88.8 ± 0.46^{a}
Fermentation time (h)	FRAP (mM $FeSO_4$)		CUPRAC (mM trolox)	
	L. acidophilus	*L. plantarum*	*L. acidophilus*	*L. plantarum*
0	5.68 ± 0.08^{a}	5.54 ± 0.06^{cd}	1.24 ± 0.04^{de}	1.26 ± 0.02^{d}
6	5.23 ± 0.08^{bc}	5.30 ± 0.11^{e}	1.29 ± 0.012^{bc}	1.23 ± 0.08^{d}
12	5.71 ± 0.06^{a}	5.54 ± 0.16^{cd}	1.58 ± 0.04^{a}	1.37 ± 0.05^{c}
18	5.24 ± 0.16^{bc}	5.73 ± 0.07^{abc}	1.34 ± 0.03^{c}	1.44 ± 0.02^{c}
24	5.00 ± 0.08^{cd}	5.87 ± 0.07^{a}	1.27 ± 0.03^{d}	1.61 ± 0.01^{ab}
30	4.81 ± 0.15^{d}	5.80 ± 0.09^{ab}	1.21 ± 0.03^{de}	1.43 ± 0.03^{c}
36	5.33 ± 0.17^{b}	5.63 ± 0.14^{bc}	1.52 ± 0.04^{b}	1.55 ± 0.01^{b}
42	5.11 ± 0.12^{bc}	5.38 ± 0.13^{de}	1.27 ± 0.03^{d}	1.68 ± 0.16^{a}
48	4.51 ± 0.16^{e}	5.74 ± 0.17^{abc}	1.16 ± 0.06^{f}	1.57 ± 0.02^{ab}

Values are expressed as the mean ± SD. Values with different letters (a ~ f) in the same column are significantly different at $p < 0.05$. A, inhibition.

Table 4: Changes in organic acid of the fermented papaya juices.

Organic acids	Papaya juice (mg/100 mL)	Fermented 48 h with *L. acidophilus* (mg/100 mL)	Fermented 48 h with *L. plantarum* (mg/100 mL)
Lactic acid	266 ± 3^{b}	543 ± 68^{a}	571 ± 32^{a}
Oxalic acid	85.7 ± 5.6^{a}	78.5 ± 7.2^{a}	80.9 ± 7.6^{a}
Tartaric acid	0.36 ± 0.03^{a}	0.10 ± 0.02^{c}	0.16 ± 0.02^{b}
Formic acid	8.81 ± 0.41^{b}	20.94 ± 4.10^{a}	18.42 ± 2.08^{a}
Pyruvic acid	0.55 ± 0.05^{b}	1.13 ± 0.15^{a}	1.20 ± 0.11^{a}
Malic acid	53.2 ± 2.8^{b}	77.6 ± 3.5^{a}	74.2 ± 2.0^{a}
Acetic acid	2.34 ± 0.13^{c}	3.86 ± 0.38^{b}	6.19 ± 0.92^{a}
Total organic acid	416	725	752

Values are expressed as the mean ± SD. Values with different letters (a ~ c) in the same row are significantly different at $p < 0.05$.

group (produces lactic acid and other products such as acetic acid) [63]. These organic acids are important secondary carbon sources for numerous microbial genera that proliferate during food fermentation. The fermentation of papaya juices resulted in a significant change in the composition of the organic acids (Table 4), and the changes in organic acids between the two cultures were similar. Lactic acid, formic acid, pyruvic acid, malic acid, and acetic acid were significantly increased after fermentation ($p < 0.05$). Lactic acid was the most abundant organic acid formed after fermentation, with *L. plantarum* producing 571.29 mg/100 mL of lactic acid. *L. plantarum* produced more acetic than *L. acidophilus* (6.19 versus 3.86 mg/100 mL). Oxalic acid did not change significantly throughout the fermentation process ($p < 0.05$). Tartaric acid, which may be used for microbial metabolism during the fermentation process, decreased significantly ($p < 0.05$) during fermentation, more with *L. acidophilus* (0.36 to 0.10 mg/100 mL) than with *L. plantarum* (0.16 mg/100 mL). Tofalo et al. [64] reported that the content of lactic acid and acetic acid increased after fermented raw milk cheese with *mesophilic lactobacilli* and *lactococci*, which is similar to our results. In contrast, Yang et al. [65] observed a decrease in the content of malic acid and acetic acid in soymilk fermented with *Bifidobacterium* and *Streptococci strains*. Lee et al. [66] reported that the acetic acid increased and citric acid remained essentially unchanged throughout the fermentation in papaya wine fermented with three *Williopsis saturnus* yeasts, which is similar to our

results. However, malic acid had a different trend in both fermentation processes.

3.4. Volatile Composition of Papaya Juices before and after Fermentation. Volatile components of various papaya cultivars have been widely studied by many scholars. More than 300 different aroma compounds have been identified in papaya fruits. Esters and alcohols are the main aroma components of papaya [67]. Various volatile components, including acids, alcohols, esters, aldehydes, ketones, and phenols, were detected in papaya juice before and after fermentation (Table 5). The volatiles present in papaya juices changed significantly after fermentation, and some differed between the two cultures.

Among the volatile acids identified, acetic acid and 3-methyl-butanoic acid were the only two volatile acids produced by both cultures. 2-Methyl-propanoic acid was newly produced by *L. plantarum*.

Alcohols were the most abundant key volatiles extracted from the fermented papaya juices. The alcohols primarily consisted of ethanol, 3-methyl-1-butanol, and linalool. Some species in the *Lactobacillus* genus has the ability to produce ethanol, having alcohol dehydrogenase enzymes that can convert acetaldehyde into ethanol [68]. 3-Methyl-1-butanol has a pleasant aroma and is a major volatile compound of cheese. The metabolic pathway for the production of 3-methyl-butanol is the catabolism of amino-acids [69]. Globally, *L. plantarum* produced more alcohols than *L. acidophilus*. In studies of the volatile components of many varieties of papaya, linalool is one of the key aromatic compounds contributors in papaya fruit [70].

Among the major volatile compounds identified, the ester compounds are the most common. A total of 25 esters were identified in papaya juice before and after fermentation (Table 5). Pino [71] reported that esters were the primary class of volatile chemical compounds in papaya fruit. Acetate esters are formed by alcohol acetyltransferases from the reaction between acetyl-CoA and alcohols. Most volatile esters can enhance fruit flavor, especially ethyl acetate [72]. A high level of ethyl acetate and butanoic acid ethyl ester (pineapple fragrance) was produced by the two cultures (Table 5).

Ketones and lactones were the next most diverse group of volatile compounds in papaya juices, both before and after fermentation (Table 5). As expected, acetone, 2-butanone, acetoin, and 6-methyl-5-hepten-2-one were dominant volatile compounds. Acetone and 2-butanone have pungent odors, and their concentrations decreased after fermentation. In contrast, the quantity of acetoin (3-hydroxy-2-butanone), which has a milk aroma, increased throughout the fermentation process. Acetoin was observed to be produced from the metabolism of citrate [73]. 2-Heptanone, 1-hydroxy-2-propanone, 2-hydroxy-3-pentanone, 2-nonanone, 2-dodecanone, and 2-tetradecanone were also produced after fermentation. They were likely derived from the β-oxidation of saturated free fatty acids and the further decarboxylation of β-ketoacids [74].

Melgarejo et al. [75] observed a negative relationship between juice quality and a high concentration of aldehydes. Aldehydes are easily reduced to alcohols or oxidized to acids in food matrices, especially in the presence of microbial activity [76]. The aldehydes became more abundant after fermentation. *L. plantarum* produced more new aldehydes than *L. acidophilus* throughout the fermentation process. Acetaldehyde and benzaldehyde were present at high levels in both FPJA and FPJP. It has been reported that benzaldehyde can provide ideal sensory properties, such as almonds, cherries, and sweetness [77]. Volatile phenols were also detected in the papaya juice before and after fermentation, and 2,4-di-tert-butylphenol was the main volatile phenol detected in the papaya juices (Table 5).

In summary, a large number of volatiles were identified in papaya beverages before and after fermentation. We observed that the aroma components were similar after fermentation but in different proportions. Their contribution to the final flavor of beverages is being further studied. Lee et al. [25] reported that, in papaya wine fermented with *Williopsis saturnus* var. *mrakii NCYC 2251*, a wide range of volatile compounds were produced during fermentation including acids, alcohols, esters, and aldehydes with esters being the most abundant volatile compounds produced. In our study, volatile compounds have similar results with papaya wine, also esters being the most abundant volatile compounds in papaya juices. The volatile profiles given by *L. acidophilus* and *L. plantarum* were quite similar, although more alcohols and aldehydes were globally found with *L. plantarum*.

4. Conclusion

The present study investigated the use of papaya beverages as the main substrate for fermentation by two lactic acid bacteria, and a strain more suitable for fermentation was selected by comparing the different physicochemical properties of fermentation. The results show that, during papaya juice fermentation, both strains produced a large amount of volatiles with generally similar changes. However, the pH decrease was 0.05 units less with *L. acidophilus* than with *L. plantarum* which was consistent with the organic acid contents, especially lactic acid. This shows that *L. plantarum* is more suitable for growth and stronger acid production capacity is based on papaya juice. Antioxidant activity tended to differ between *L. acidophilus* and *L. plantarum*. The antioxidant capacity of FPJA decreased significantly, whereas the antioxidant capacity of FPJP increased after fermentation, showing a better oxidation resistance. Through comparisons of the pH, organic acids, antioxidant components, and volatile compounds, especially the change in antioxidant activity, it was found that *L. plantarum* is more suitable for the production of fermented papaya beverages. It is worth noting that both FPJA and FPJP produce a large number of flavonoids, which has become a hot research topic in recent years because of its pharmacological activity. We will conduct additional relevant tests to study the efficacy of LAB fermentation of papaya, such as the effect of weight loss and lipid lowering. In a word, fermented papaya juice could be a novel probiotic beverage for consumers.

Table 5: Major volatile compounds and relative peak area in papaya juices before and after 48 h fermentation.

Volatile compounds	RT	RI	Papaya juice PA × 10^6	FPJA 48 h PA × 10^6	FPJP 48 h PA × 10^6
Acids					
Acetic acid	19.36	1444	319.80 ± 17.04^c	1809.78 ± 80.37^a	753.41 ± 20.64^b
Propanoic acid	21.81	1537	23.48 ± 3.56^a	–	–
2-Methyl-propanoic acid	22.56	1567	–	–	28.15 ± 1.55^a
Butanoic acid	23.94	1623	157.19 ± 9.48^b	195.87 ± 11.58^a	195.54 ± 8.36^a
3-Methyl-Butanoic acid	24.97	1666	–	55.83 ± 2.64^b	228.29 ± 11.57^a
2-Methyl-Butanoic acid	25.00	1668	–	–	199.49 ± 20.60^a
Hexanoic acid	28.93	1845	99.80 ± 5.94^a	–	–
2-Ethyl-Hexanoic acid	31.15	1953	17.54 ± 1.21^a	16.76 ± 1.75^a	–
Octanoic acid	33.33	2063	105.10 ± 6.35^b	104.05 ± 5.36^b	147.15 ± 9.17^a
Nonanoic acid	35.45	2174	25.18 ± 2.81^b	32.54 ± 1.27^a	–
n-Decanoic acid	37.44	2281	22.54 ± 1.38^a	–	–
Alcohols					
Ethanol	4.28	930	475.70 ± 29.14^c	5070.47 ± 156.45^a	3306.28 ± 180.67^b
1-Propanol	7.11	1043	–	15.78 ± 2.36^b	23.35 ± 1.48^a
2-Methyl-1-propanol	9.14	1113	–	–	43.67 ± 3.50^a
1-Butanol	10.71	1160	–	7.00 ± 0.27^b	27.01 ± 2.36^a
1-Penten-3-ol	11.14	1173	8.86 ± 0.35^a	2.62 ± 0.13^c	3.78 ± 0.05^b
3-Methyl-1-butanol	12.66	1219	12.45 ± 1.50^c	1107.19 ± 42.15^b	1542.59 ± 37.75^a
3-Methyl-3-buten-1-ol	13.84	1255	–	–	15.17 ± 2.58^a
1-Pentanol	13.96	1259	9.53 ± 1.32^b	–	18.93 ± 1.86^a
Prenol	16.00	1325	–	10.01 ± 0.83^a	12.64 ± 2.87^a
3-Pentanol	16.43	1340	–	29.02 ± 1.48^b	46.42 ± 3.40^a
1-Hexanol	16.95	1357	12.44 ± 2.74^c	38.82 ± 1.39^b	51.03 ± 3.65^a
1-Heptanol	19.77	1459	8.50 ± 0.46^a	5.92 ± 0.27^c	7.43 ± 0.24^b
2-Ethyl-1-hexanol	20.69	1493	836.35 ± 32.85^a	–	–
Linalool	22.04	1546	944.70 ± 74.91^a	600.17 ± 27.61^c	766.51 ± 30.67^b
1-Octanol	22.39	1560	22.64 ± 3.67^c	56.57 ± 3.40^a	33.45 ± 1.42^b
1-Nonanol	24.82	1660	40.83 ± 7.36^a	–	39.66 ± 5.82^a
1-Decanol	27.15	1762	–	42.84 ± 4.16^a	17.81 ± 1.27^b
Geraniol	28.94	1845	–	–	97.05 ± 7.43^a
Benzyl alcohol	29.44	1869	19.58 ± 0.97^c	90.93 ± 7.34^b	133.85 ± 8.61^a
Phenylethyl alcohol	30.20	1905	–	117.29 ± 6.97^b	222.19 ± 21.85^a
Nerolidol	32.83	2037	–	–	2.12 ± 0.03^a
Aldehydes					
Acetaldehyde	2.05	6956	370.89 ± 23.64^a	287.47 ± 12.34^b	368.09 ± 15.28^a
Pentanal	5.19	970	–	–	84.82 ± 6.34
Hexanal	8.29	1085	–	–	12.47 ± 0.72
2-Methyl-2-heptenal	10.11	1142	20.95 ± 1.78^a	–	21.67 ± 1.81^a
Heptanal	11.66	1188	–	–	24.97 ± 0.94^a
Octanal	14.93	1289	–	–	30.47 ± 4.35^a
2,6-Dimethyl-5-heptenal	16.80	1352	2.79 ± 0.08^a	–	–
Nonanal	17.96	1392	104.87 ± 2.65^b	49.84 ± 10.45^c	153.20 ± 6.45^a
Benzaldehyde	21.24	1514	27.89 ± 1.84^b	27.53 ± 1.63^b	57.56 ± 2.61^a
Dodecanal	25.90	1706	–	9.13 ± 0.27^b	19.50 ± 0.88^a
2,4-Dimethyl-benzaldehyde	28.02	1801	–	3.58 ± 0.80^b	26.92 ± 1.47^a

Table 5: Continued.

Volatile compounds	RT	RI	Papaya juice PA × 10^6	FPJA 48 h PA × 10^6	FPJP 48 h PA × 10^6
Esters					
Acetic, methyl ester	2.74	820	6.97 ± 0.07^b	4.66 ± 0.26^c	11.62 ± 0.62^a
Acetic, ethyl ester	3.38	881	3.93 ± 0.12^b	42.89 ± 3.90^a	37.62 ± 3.55^a
Butanoic acid, methyl ester	5.41	979	11.35 ± 0.58^a	3.35 ± 0.21^c	7.23 ± 0.74^b
Butanoic acid, ethyl ester	6.96	1038	–	35.88 ± 1.54^a	18.37 ± 0.93^b
Hexanoic acid, methyl ester	11.77	1191	9.41 ± 1.40^a	–	2.77 ± 0.07^b
Hexanoic acid, ethyl ester	13.31	1239	–	8.55 ± 1.30^a	–
Thiocyanic acid, methyl ester	14.22	1267	7.01 ± 0.65^a	3.09 ± 1.32^b	4.29 ± 0.68^b
Acetic acid, 2-ethylhexyl ester	17.75	1385	6.54 ± 1.28^a	3.15 ± 0.47^b	5.82 ± 0.85^a
Octanoic acid, methyl ester	17.89	1390	48.07 ± 3.67^a	5.55 ± 0.24^c	11.89 ± 1.52^b
Octanoic acid, ethyl ester	19.14	1436	9.88 ± 0.42^b	16.59 ± 1.37^a	10.12 ± 0.73^b
n-Butyric acid 2-ethylhexyl ester	21.44	1522	207.11 ± 10.37^c	227.53 ± 5.39^b	255.75 ± 6.74^a
Decanoic acid, methyl ester	23.23	1593	75.10 ± 3.43^a	14.32 ± 1.50^b	10.17 ± 0.64^c
Decanoic acid, ethyl ester	24.27	1637	9.93 ± 0.27^b	13.59 ± 0.64^a	9.30 ± 0.55^{bc}
Hexanoic acid, 2-ethylhexyl ester	25.97	1709	60.19 ± 5.39^a	50.84 ± 3.276^b	60.42 ± 3.46^a
Dodecanoic acid, methyl ester	27.98	1799	33.23 ± 1.27^a	20.09 ± 2.35^b	9.92 ± 3.57^c
Dodecanoic acid, ethyl ester	28.88	1842	2.75 ± 0.06^b	4.54 ± 0.61^a	2.62 ± 0.24^b
Tetradecanoic, methyl ester	32.28	2009	36.71 ± 1.85^a	21.28 ± 3.65^b	–
Myristoleate, methyl ester	33.02	2047	13.54 ± 0.64^a	–	–
Tetradecanoic acid, ethyl ester	33.06	2049	–	9.64 ± 0.15^a	–
Benzoic acid, 2-ethylhexyl ester	35.38	2171	24.12 ± 1.34^a	21.47 ± 0.76^b	19.18 ± 0.51^c
Hexadecanoic acid, methyl ester	36.22	2216	6.72 ± 2.68^a	–	–
Dodecanoic acid, ethyl ester	36.90	2252	–	8.71 ± 0.34^a	–
9-Hexadecenoate, ethyl ester	37.37	2277	37.39 ± 6.37^a	7.61 ± 0.69^b	–
1,2-Benzenedicarboxylic acid, bis(2-methylpropyl) ester	41.66	2506	–	32.24 ± 2.31^a	23.12 ± 3.16^b
Dibutyl phthalate	44.09	2636	–	12.25 ± 3.20^a	9.71 ± 0.91^b
Ketones and lactones					
Acetone	2.62	809	483.80 ± 32.45^a	411.31 ± 16.38^b	410.72 ± 11.27^b
2-Butanone	3.55	897	234.15 ± 11.84^a	146.93 ± 14.75^b	166.03 ± 20.84^b
2,3-Butanedione	5.16	968	107.29 ± 6.87^a	113.63 ± 8.55^a	84.82 ± 6.98^b
2-Heptanone	11.61	1187	–	–	25.26 ± 5.27^a
Acetoin	14.79	1285	752.58 ± 55.28^c	2804.69 ± 96.38^b	1688.42 ± 67.38^a
1-Hydroxy-2-Propanone	15.15	1296	–	–	4.35 ± 0.76^a
6-Methyl-5-Hepten-2-one	16.36	1337	276.58 ± 15.94^a	139.80 ± 23.70^b	79.95 ± 11.56^c
2-Hydroxy-3-pentanone	16.91	1356	–	38.81 ± 2.85^b	51.03 ± 3.44^a
2-Nonanone	17.86	1389	–	20.28 ± 1.74^a	–
3-(Hydroxymethyl)-2-Nonanone	17.92	1391	48.07 ± 2.95^a	50.78 ± 3.64^a	4.79 ± 0.12^b
2-Octanone	23.30	1596	5.60 ± 0.14^c	20.43 ± 1.38^a	11.85 ± 1.30^b
2-Dodecanone	28.12	1806	–	5.63 ± 0.47^a	–
2-Tetradecanone	28.12	1806	–	–	8.03 ± 1.14
Phenols					
2,6-Bis(1,1-dimethylethyl)-4-(1-oxopropyl)phenol	30.52	1921	–	14.33 ± 0.92^a	14.22 ± 1.17^a
Phenol	31.98	1994	–	43.80 ± 4.51^a	26.82 ± 3.95^b
2,4-Di-tert-butylphenol	37.78	2299	84.52 ± 6.87^a	45.58 ± 3.63^b	36.54 ± 3.29^c
2,6-Di-tert-butyl-4-methoxyphenol	46.52	2765	5.44 ± 1.23^a	–	–

Values are expressed as the mean ± SD. Values with different letters (a ~ c) in the same row are significantly different at $p < 0.05$. RT, retention time; RI, retention index; PA, peak area; RPA, ratio of peak area; "–", not detected.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

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