

Research Article

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Effect of *Lactiplantibacillus plantarum* and prebiotics on physicochemical, **microbiological, and sensory quality of pasteurized raw mango juice**

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ABSTRACT

This study aimed to develop a functional beverage from a local mango variety, "Kaew," and the potential probiotic *Lactiplantibacillus plantarum* PW1 (*Lp*) enriched with prebiotics, including fructooligosaccharides, inulin, and polydextrose. The effects of *Lp* and these functional ingredients on the beverage's physicochemical, microbiological, and sensory qualities after fermentation and during refrigeration were investigated. The addition of the potential probiotic *Lp*, along with prebiotics, was effectively applied in ready-to-drink raw mango juice. The viability of the *Lp* strain remained above 7 Log CFU/mL throughout the study periods. The findings highlighted the potential to enhance the value of mango juice through fermentation with the beneficial bacteria *Lp*, combined with exogenous prebiotics.

Keywords: mango juice*, Lactiplantibacillus plantarum,* prebiotic

INTRODUCTION

Mango (*Mangifera indica* L.) is one of the world's most popular tropical fruits widely produced and consumed (Rumainum et al., 2018). Mango is rich in essential nutrients, including carbohydrates, lipids, fatty acids, proteins, amino acids, organic acids, vitamins, and minerals (Lebaka et al., 2021). Additionally, it contains various functional compounds, such as pectin, phenolic acids, flavonoids, and pigments. (Maldonado-Celis et al., 2019). Thailand is a leading producer and exporter of mangoes, with a production of 1.4 million tons in 2022 (Worldpopulationreview, 2024), and it became the world's second-largest exporter of mangoes in 2023 (FAO, 2024). Thailand has a vast diversity of mango cultivars, with over 100 distinct varieties cultivated throughout the country (Pott et al., 2004). Among these, the Nam Dok Mai cultivar is particularly popular in commercial markets due to its unique aromatic flavor. Additionally, local cultivars offer various options to satisfy consumer preferences and market demands. Notably, the Kaew cultivar, grown in different regions of Thailand, is favored for fresh consumption due to its distinct flavor and texture (Horticultural Research Institute, 2024). Additionally, it plays a vital role in the pickle mango industry (Paunrat et al., 2024). However, mangoes typically ripen after harvesting when stored at room temperature for 2-10 days, softening to an acceptable quality before deteriorating (Kumar et al., 2023). Therefore, expanding the use of various food

processing techniques can provide an alternative for preservation. Moreover, it can enhance the value of oversupplied and sub-grade mangoes during the season.

Fruit-based functional beverages are gaining popularity due to their richness in bioactive compounds (Laophongphit et al., 2024). These beverages can be formulated with probiotics and prebiotics to increase health benefits. International Scientific Association for Probiotics and Prebiotics (ISAPP) defines prebiotics as a substrate that is selectively utilized by host microorganisms, conferring a health benefit, probiotics as live strains of strictly selected microorganisms which, when administered in adequate amounts, confer a health benefit on the host (Gibson et al., 2017). Using Kaew mango to develop functional beverages can meet consumer preferences for healthier dietary choices while significantly enhancing their value and promoting the conservation of biological diversity. This approach aligns economic interests with environmental sustainability, creating a win-win situation for farmers, consumers, and producers.

Prebiotics offer an exciting novel dietary management approach for various aspects of health, particularly gut health. Thus, they are extensively applied in the food industry. Fructooligosaccharides (FOS), inulin, and polydextrose (PDX) are prebiotics that have been extensively reported for use in various food products, including beverages (Renuka et al., 2009; Carmo et al., 2016; White and Hekmat, 2018;

Fakhri et al., 2023). The chemical structure of inulin consists of linear chains of fructose units ranging in length from 2-60 (Afinjuomo et al., 2021). It provides 25–35% of the energy of digestible carbohydrates and has a sweetness level of 10% of that of sucrose (Richardson et al., 2021). PDX is a highly branched, randomly bonded glucose polymer with an average DP of 12, ranging from 2-120. It is not sweet and has a neutral taste, providing approximately 1 kcal/g of energy through partial fermentation by the microbiota (Carmo et al., 2016). FOS is a short-chain sugar composed of a single glucose molecule bonded to 2-4 additional fructose molecules (USDA, 2015), with properties similar to sucrose but providing only about 30–50% of its sweetness (Renuka et al., 2009; Hughes et al., 2021).

These prebiotics promote several health benefits, particularly for gut health, and their ability to enhance the nutritional profile of foods makes them valuable ingredients in the food industry. They are substances that cannot be digested by the human body but are fermented by beneficial intestinal flora, helping them compete with harmful species and promoting the production of beneficial fermentation products such as short-chain fatty acids (SCFAs), including acetate, butyrate, and propionate (McLoughlin et al., 2017; You et al., 2022; Chin et al., 2024).

Lactiplantibacillus plantarum (*Lp*) is one of the most commonly used commercial starters for the fermentation of plant foods and probiotics (You et al., 2022; Munoz et al., 2024; Ribera et al., 2024). The preliminary study indicated that *Lp* has potential as a probiotic compared to the other tested strains. In the present study, the development of a functional beverage from a native mango, "Kaew," and *Lp* enriched with prebiotics, FOS, inulin, and PDX was explored. For this purpose, the effects of *Lp* and functional ingredient use on physicochemical, microbiological, and sensory quality were investigated after fermentation and during refrigeration

MATERIALS AND METHODS

Raw material

A Thai cultivar, Kaew, was obtained from the local market in Lampang province, Thailand. In this study, the raw mango fruits were sorted at the mature-green stage and used for juice production. Three food-grade prebiotics, FOS, inulin, and PDX, were purchased from Bangkok Chemical Co., Ltd., Thailand.

Preparation of juice

After washing, the mango fruits were manually peeled, and the flesh was separated from the seeds. The flesh was then processed using a juice

extractor (Tefal model ZC420E38) to obtain the juice. The mango juice (MJ) was formulated to achieve a final composition of 20% (w/w) juice extract, 5% (w/w) prebiotics (FOS, inulin, and PDX), and table sugar added to reach a total soluble solids (TSS) of 15°Brix. The formulated juice was then filtered through an 80-mesh nylon cloth. Mango juice without adding prebiotics was used as a control (MJC). The juice was then pasteurized at 80-85°C for 10 minutes and cooled to 35°C.

Mango juice fermentation

The *Lp* was cultured in MRS broth at 30°C for 24 h to prepare the inoculum. After centrifugation at $8000 \times g$ for 15 minutes, the cells were washed twice with sterile deionized (DI) water and resuspended in the pasteurized mango juice. An inoculum of L_p , at a concentration of 2×10^7 CFU/ml, was added to four pasteurized mango fruit juices (Table 1). Fermentation was conducted at 35°C for 24 h; after that, the fermented mango juice samples were stored in the refrigerator at 4°C.

Table 1. Fermentation treatment for pasteurized raw mango juice

Treatment	Bacterial strain	Prebiotic		
MJC	-	-		
MJL.	Lp	-		
MJLF	Lp	FOS		
MJLI	Lp	Inulin		
MJLP	Lp	PDX		

 $MJC = control; MJL = MJ + Lp; MJLF = MJ + Lp + FOS; MJLI$ $= MJ + Lp + \text{inulin}$; $MJ = MJ + Lp + \text{PDX}$; $MJ = \text{mango juice}$.

Microbiological analysis

Enumeration of *Lp* and fungi (yeast and mold) from all mango juice samples was carried out for 0 and 24 h fermentation and during the storage period (7 and 14 days). MRS and PDA media were used to determine *Lp*, yeast, and mold count, respectively. The plates were incubated anaerobically at 37°C for 48 h and 25°C for 7 days, respectively. Viable counts were analyzed and recorded as colonyforming units per mL (CFU/mL).

Bioactivity assay

The effects of FMJ samples on the populations of lactic acid bacteria (LAB) and pathogenic bacteria were evaluated according to Wongputtisin and Khanongnuch (2015). In brief, the LABs used in this experiment were *Lactobacillus acidophilus*, *Lactobacillus bulgaricus, Lactobacillus lactis,* and *Lp. plantarum*. Normal flora and pathogens used were *Escherichia coli*, *Bacillus cereus, Staphylococcus aureus*, *Salmonella* Enteritidis, and *Salmonella* Typhimurium. The sample (50 g/L) was used as a carbon source in basal medium containing (g/L) : K₂HPO₄ 0.3, KH₂PO₄ 0.1,

yeast extract 1.0, peptone 1.0, MgSO4 0.2 and $(NH_4)_2SO_4$ 2.5. The 24 h-old inoculum (10⁷ CFU) of tested strains were prepared and separately inoculated. Viable counts were analyzed and recorded as colony-forming units per mL (CFU/mL). Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) were determined according to Jitpakdee et al. (2022) and Rodríguez-Melcón et al. (2022). In brief, FMJ samples were tested in four bacteria cultures of *E. coli*, *Bacillus cereus, S. aureus*, *S.* Enteritidis, and *S.* Typhimurium using the two-fold dilution technique. MIC values were taken as representative of the lowest FMJ juice concentration that prevented visible bacterial growth after 24 h of incubation at 37°C, and the MBC was representative of the lowest concentration that completely inhibited bacterial growth.

Chemical analysis

All mango juice samples were measured for pH by a digital pH meter (Model C831, Belgium). The total acidity contents of the samples were determined by diluting each 10 ml aliquot of sample in 20 ml distilled water and then titrating to pH 8.2 using 0.1 N NaOH (Iland et al., 2000). Titratable acidity was expressed as citric acid percentages. The Atago hand-held refractometer was used to determine TSS in the samples. Reducing sugar contents (RS) in the samples were quantified by Rebelein method (Iland et al., 2000). The Folin-Ciocalte method was used to determine total phenolic contents in the samples (Spínola et al., 2015). The antioxidant capacity was determined by the ABTS assay and expressed as vitamin C and Trolox equivalent antioxidant capacity in mg/100 mL of sample (Wongputtisin et al., 2007). An average of three replicates of each treatment was taken.

Sensory analysis

All the panelists had experience with fruit juices. A group of 20 panelists participated in this study; they evaluated the sensory quality of FMJ samples after 24 h and following 14 days of refrigeration storage. The samples were served randomly at 4°C in plastic cups labeled with a random three-digit code. Panelists were instructed to rinse their mouths with water between samples. Each panelist assessed five samples of fermented mango juice, providing feedback on sensory characteristics. They evaluated the appearance, color, odor, flavor, and overall preference of the final product using a nine-point hedonic scale, ranging from 9 (extremely liked) to 1 (extremely disliked) for each characteristic (Meilgaard et al., 2006).

Sensory analysis

Results are expressed by mean ± standard deviation (SD) of 3 replications. Data were analyzed for significant differences using analysis of variance

(ANOVA). Duncan's new multiple range test was used to analyze pairwise comparisons of the means when there was a significant difference ($P \le 0.05$). All the panelists had experience with fruit juices. A group

RESULTS AND DISCUSSION

This study explored an alternative use of the native Kaew mango cultivar for beverage production. By adding value to mango juice with the *Lp*, enriched with commercial prebiotics, the results highlight the feasibility of producing beverages from this native mango cultivar.

Microbiological properties of fermented mango juices

LAB can produce fermented beverages from various fruits, including grapes, oranges, pineapples, apples, apricots, and bananas (Pinto et al., 2022). This study investigated the effects of *Lp* in combination with different prebiotics on the fermentation of pasteurized raw MJ. Viable counts of LAB indicated that *Lp* could grow and survive in the juices after 24 hours of fermentation, with counts ranging from 7.63-7.78 CFU/mL. These levels meet the recommended probiotic viable cell counts of 6-11 log CFU/mL, ensuring the juices provide health benefits (Papun et al., 2024). Considering the prebiotic types in this study, the highest LAB population was found in FMJ supplemented with inulin ($P < 0.05$). Additionally, no growth of yeast or mold was detected in any of the tested MJ samples (Table 2).

Means in the same column with different superscripts indicate significant differences ($P \le 0.05$). n.d. = not detected. MJC = control; MJL = $MJ + Lp$; MJLF = $MJ + Lp$ + FOS; MJLI = $MJ +$ Lp + inulin; MJLP = $MJ + Lp$ + PDX; MJ = mango juice.

Bioactivity properties of MJ

Effects of MJ with different treatments on the growth of tested bacteria were performed. Glucose is a monosaccharide commonly utilized by various microorganisms (Chen et al., 2023; Zhang et al., 2023), resulting in the observed growth of LAB and pathogenic bacteria in this study (Figure 1). The results indicated that both prebiotic-added and nonadded FMJs influenced the growth of the test bacteria differently. FMJs containing the three prebiotics

promoted the growth of the tested LAB, except for *L. acidophilus*, which showed reduced growth in FMJs with added FOS and inulin. These results align with previous studies that have shown that different prebiotics support varying levels of bacterial growth (Geng et al., 2023; Ju et al., 2024).

Considering bacterial growth in a basal medium with glucose as the carbon source, some LAB and pathogenic bacteria exhibited better growth compared to the medium with the addition of the tested mango juice samples. That is not consistent with the definition of a prebiotic (Gibson et al., 2017). This occurrence may be influenced by various factors, including juice components, pH, and temperature during processing, which can affect prebiotic stability (Glibowski and Bukowska, 2011; Duar et al., 2015; Mensink et al., 2015; Fonteles and Rodrigues, 2018; Arruda et al., 2020). Therefore, further investigation into the changes in prebiotics

under the conditions of this study is necessary. Additionally, growth can also be influenced by the different strains of the tested bacteria (Fratianni et al., 2023; Lee et al., 2023; Moon et al., 2024).

In practical use, some portion of glucose may be utilized by pathogens and other bacteria colonizing the digestive tract, leaving less carbon source available for probiotics. Additionally, glucose is typically absorbed and utilized by the host before it reaches the colon. When growth in basal medium supplemented with the FMJ samples was considered, the growth of LAB was promoted. At the same time, pathogens and normal flora were lower, indicating that the FMJ samples prepared from various prebiotics were utilized less efficiently by these pathogens than glucose. This might demonstrate the positive effect of the prebiotic properties of the FMJ samples.

Figure 1. Microbial population changes in basal medium, basal medium supplemented with glucose and basal medium supplemented with various mango juice samples; lactic acid bacteria (a) and pathogenic bacteria (b). MJC = control; MJL = MJ + *Lp*; MJLF = MJ + *Lp* + FOS; MJLI = MJ + $Lp + \text{inulin}$; MJLP = MJ + $Lp + \text{PDX}$; MJ = mango juice.

Inhibitory effects of MJ

The ability of the FMJ samples to inhibit the growth of five food-borne pathogenic bacteria at MIC and MBC levels in a range of the FMJ samples diluted at 62.5-990 µL/mL is presented in Table 3. The MICs varied notably depending on different

samples and bacteria species. The values $(\mu L/mL)$ for MICs ranged from 124–495 µL/mL for MGJ, 124– 495 µL/mL for MGJL, and 124-248 µL/mL for MGJLF and MGJLI, while the values for MICs of MGJLP was 124 µL/mL. The MJ and FMJ samples exhibited the highest inhibitory activity against *B.*

cereus, with MIC and MBC values of 124 and 248 µL/mL, respectively. The values for MBCs were greater than or equal to those for MICs across all tested species. Moreover, considerable differences were observed between strains. The recorded values for MBCs (μ L/mL) ranged from 248 to no inhibition for all tested samples. FMJ samples inhibited bacteria better than MGJ, MGJLF, and MGJLI, demonstrating higher efficacy than MGJL and MGJLP. However, variation of MBCs depending on different samples

and bacteria species was observed. The antimicrobial activity of FMJ samples can result from various metabolites produced by *Lp*, including organic acids and other antimicrobial compounds such as bacteriocins (Peng et al., 2020; Jeong et al., 2024). This result suggests that MGJ fermented by *Lp* and with a prebiotic supplement has great potential as a functional beverage that might inhibit pathogenic bacteria causing food-borne diseases.

Table 3. Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) for various mango juice samples on five species of bacteria

Antibacterial properties	Treatment	E. coli	B. cereus	S. aureus	S. enteritidis	S. typhimurium
MIC (µL/mL)	MJC	495	124	248	495	495
	MJL	495	124	124	124	124
	MJLF	124	124	248	124	248
	MJLI	248	124	124	248	124
	MJLP	124	124	124	124	124
$MBC(\mu L/mL)$	MJC	495	248	$\qquad \qquad$	-	-
	MJL	$\qquad \qquad -$	248	$\qquad \qquad -$	495	495
	MJLF	250	248	248	495	495
	MJLI	495	248	495	495	495
	MJLP	495	248	$\overline{}$	-	495

 $-$ = no inhibitory activity at the tested maximum concentration of 990 $\mu L/mL$. MJC = control; MJL = MJ + *Lp*; MJLF = MJ + *Lp* + FOS; MJLI = $MJ + Lp$ + inulin; MJLP = $MJ + Lp$ + PDX; MJ = mango juice.

Chemical properties of fermented mango juices

The effect of *Lp* on the chemical properties of fermented and unfermented mango juice samples is presented in Table 4. The FMJ samples with *Lp* resulted in lower pH values and higher acidity. This performance was aligned with the contents of total soluble solids and reducing sugars. The counts of *Lp* in FMJ samples indicated its activity, which influenced the quality of FMJ samples. This observation could be explained by the characteristics of *Lp* belonging to LAB that transform carbohydrate substrates into organic acids, including lactic acid, formic acid, acetic acid, and propionic acid, creating unfavorable conditions for the growth of spoilage and pathogenic microorganisms (Park et al., 2019; Bangar et al., 2022). Additionally, LAB consumes

sugars, producing nonvolatile acids and volatile aroma compounds that preserve products while creating unique flavors, textures, and enhanced nutritional profiles (Kayitesi et al., 2023). Other components secreted from the metabolism of *Lp* during the fermentation of MJ may play a significant role in its functional properties. These properties could also be linked to postbiotics, as described by Siciliano et al. (2021). Furthermore, certain compounds in fruit juices may exhibit prebiotic properties and promote the growth of LAB (Mantzourani et al., 2024). Thus, the FMJs, with the addition of the prebiotics from this study, could potentially provide a synbiotic effect by combining probiotics and prebiotics that beneficially impact the host (Ribera et al., 2024). However, further investigation is needed to support health claims.

Means in a column with the different superscripts represent significant differences ($P \le 0.05$). TA = Titratable acidity (% as lactic acid); TSS = total soluble solids (°Brix); $RS =$ reducing sugar (mg/L). MJC = control; MJL = MJ + *Lp*; MJLF = MJ + *Lp* + FOS; MJLI = MJ + *Lp* + inulin; MJLP = $MJ + Lp + PDX$; MJ = mango juice.

The FMJ samples showed higher values of antioxidant capacity and phenolic content compared with unfermented mango juice samples (Table 5). These beneficial effects may be linked to the phenolic acids found in fruits and vegetables, which are primarily present in conjugated forms such as glycosides, esters, or polymers and are covalently bound to plant structures like cellulose, hemicellulose, lignin, and pectin, as well as to structural proteins (Munoz et al., 2024). The biotransformation of these phenolic complexes during food processing or the metabolic activity of LAB facilitates the release of phenolic compounds. Similarly, lactic acid fermentation of other fruit juices

enhanced total phenolic contents in the fermented juice samples (Cele et al., 2022; Mantzourani et al., 2024). In addition, the effects of *Lp* and prebiotics in the FMJ samples were detected. The antioxidant capacity values in various FMJ samples did not vary with the values of total phenolic contents. This phenomenon may explain the fact that phenolic compounds are typically the main contributors to antioxidant activity in terms of radical scavenging and cannot entirely predict the DPPH or/and ABTS percentage since vitamin C and carotenoids also partially contribute to antioxidant functions (Mantzourani et al., 2024).

Means in a column with the different superscripts represent significant differences ($P \le 0.05$). AOA = antioxidant capacity as ascorbic acid equivalent; AOT= antioxidant capacity as Trolox equivalent; TPC = total phenolic content. MJC = control; MJL = \dot{M} + Lp ; MJLF = MJ + $Lp + FOS$; MJLI = MJ + $L\vert p$ + inulin; MJLP = MJ + Lp + PDX; MJ = mango juice.

Microbial and chemical changes of fermented mango juices during refrigeration storage

FMJ samples exhibited viable counts of LAB during two weeks of storage at 4°C, ranging from 7. 53-7.78 CFU/mL (Figure 2a). The pH values of uninoculated MJ (MJC) were higher than those of inoculated MJs with the addition of prebiotics, which aligns with the observed increase in total acidity (Figure 2b and c). These results indicate that the prebiotics used in this study could enhance acid production by *Lp* during MJ fermentation. The influence of various prebiotics on lactic acid production and pH reduction has also been reported in fermented vegetables (Zhao et al., 2024).

The total soluble solid contents of the FMJ samples during refrigeration storage fluctuated slightly between 14.40 and 15.03°Brix (Figure 2d). This variability may result from the complex matrix of the juices, including metabolites released by *Lp*, absorbed compounds, and biochemical reactions occurring within the juices.

Variability in reducing sugars, phenolic content, and antioxidant activity in the FMJ samples during storage was observed (Figure 3). This variability can be attributed to several factors influencing the chemical compounds in the juices. For instance, sugars and phenolic acids may be released through bacterial enzymes and the metabolism of sugars by bacteria (Cele et al., 2022; Munoz et al., 2024).

Figure 2. Changes in lactic acid bacteria (a); pH (b); titratable acidity (c); and total soluble solids (d) in various mango juice samples during storage at 4° C for 2 weeks. MJC = control; MJL = MJ + *Lp*; MJLF = MJ + *Lp* + FOS; MJLI = MJ + *Lp* + inulin; MJLP = MJ + *Lp* + PDX; MJ = mango juice.

Figure 3. Changes in reducing sugar contents (a); total phenolic content (b); AOA = antioxidant activity equivalent with ascorbic acid (c); and (d) AOT = antioxidant activity equivalent with trolox in various mango juice samples during storage at 4° C for 2 weeks. MJC = control; MJL $= MJ + Lp$; $\text{MJLF} = MJ + Lp + \text{FOS}$; $\text{MJLI} = MJ + Lp + \text{inulin}$; $\text{MJLP} = \text{MJ} + Lp + \text{PDX}$; $\text{MJ} = \text{mango juice}$.

Sensory properties of fermented mango juices

The hedonic scores of all tested FMJ samples were evaluated by an experienced panel. The hedonic results of five attributes (appearance, color,

odor, flavor, and overall preference) were not significantly affected ($P > 0.05$) by the application of *Lp* and various prebiotics in the samples (Figure 4).

Figure 4. The hedonic scores (mean ± sd) for sensorial attributes of various mango juice samples after fermentation (a); and after 14 days of storage (b); ns on bar graphs indicate that there was no significant difference $(\overrightarrow{P} > 0.05)$. MJC = control; MJL = MJ + *Lp*; MJLF = MJ + Lp + FOS; MJLI = MJ + Lp + inulin; MJLP = MJ + Lp + PDX; MJ = mango juice.

The sensorial testing showed that the average hedonic scores of overall preference for all tested FMJ samples were between like slightly and like moderately. Although the treatments with the potential probiotic and prebiotic supplement did not significantly affect sensory properties, the control aspect of acid production and other positive compounds led to the quality of the beverage concerning food safety, functional benefit, and its unique product.

CONCLUSIONS

The findings highlighted the potential of adding value to MJ by fermenting it with the beneficial bacterium Lp , supplemented with beneficial bacterium *Lp*, supplemented with commercial prebiotics. Although further investigation is needed, fermented MJ supplemented with inulin in this study appears to support the growth and survival of *Lp*. Therefore, the combination of LAB and prebiotics can be an alternative to enhance the health benefits and potential market appeal of beverages made from the Kaew mango cultivar.

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