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Research Article

Comparative Analysis of *Lactobacillus* spp. Fermentation in Five Fruit Drinks: Impacts on Lactic Acid Production and Cell Viability

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ABSTRACT

Fruit drinks, which contain at least 5% fruit juice and are typically non-fermented, provide a promising base for developing non-dairy functional beverages. Fermenting these drinks with lactic acid bacteria (LAB), recognized as safe for consumption, could enhance their health benefits and functionality. This study aimed to assess the lactic acid production and cell viability of different *Lactobacillus* spp. during the fermentation of fruit drinks. Five *Lactobacillus* spp., namely *Lacticaseibacillus paracasei*, *Lactiplantibacillus plantarum, L. acidophilus, Lacticaseibacillus rhamnosus*, and *LimosiLactobacillus reuteri* were utilized to ferment five different fruit drinks. Results show that *L. plantarum* exhibited superior cell growth and viability, with lactic acid production comparable to the other *Lactobacillus* spp.. Moreover, different *Lactobacillus* strains were found to produce varying concentrations of lactic acid across different fruit juices. This study demonstrates the viability of probiotics in fruit drinks, paving the way for the development of functional beverages with potential benefits for gut health and overall well-being.

Key words: Fermentation, fruit drink, probiotic, growth profile, cell viability, lactic acid

INTRODUCTION

Fermentation is a natural process in which sugars are converted into organic acids. Lactic acid bacteria (LAB), primarily from the genera *Lactobacillus, Streptococcus,* and *Leuconostoc*, play a crucial role in food fermentation (Rezac *et al*., 2018). Probiotics are live microbial supplements that, when consumed in sufficient quantities, improve gut microflora balance and provide health benefits such as antimicrobial effects against pathogens, anti-tumor properties, immunomodulation, and aid in managing cholesterol, diabetes, diarrhea, and lactose intolerance (Petrariu *et al*., 2024). Fermented foods that are commercially produced often serve as carriers for probiotic bacteria. LAB must possess certain phenotypic traits to grow efficiently in fruit juices, enhancing their safety as well as their nutritional and sensory value *(Garcia et al*., 2020).

Dairy food is an excellent source of beneficial bacteria such as *Lactobacillus spp.* since these foods improve the chance of bacteria's survivability in the intestine and buffer the stomach acid (Aljutaily *et al*., 2020). Nevertheless, some people have to avoid this dairy food, which is because some of them are vegetarian, cannot digest lactose, and are allergic to proteins (Nguyen *et al*., 2019). For that reason, they need a suitable carrier of probiotics for them to reap the benefits of those beneficial bacteria.

Fruit juices fortified with probiotics form a novel category of functional foods by generating various bioactive compounds, thereby enhancing nutritional properties and offering health benefits (Žuntar *et al*., 2020). With a rapidly expanding market driven by new sociodemographics that frequently incorporate sustainable concepts of food production, the functional food sector is the most lucrative segment of the food industry *(Putnik et al*., 2020).

There has been a significant increase in the demand for non-dairy probiotic products as a substitute for dairy probiotic foods (Prado *et al*., 2008). The application of probiotic cultures in non-milk products is challenging for scientists (Goderska *et al*., 2007). An understanding of the effects on the survival of probiotics during fermentation processes needs further studies. In this study, the effects of five different *Lactobacillus* spp. strains on growth profile, lactic acid production, and their viability in the fermentation of fruit drinks were studied for the development of a future functional beverage.

MATERIALS AND METHODS

Sample collection and preparation of the *Lactobacillus* spp. starter culture.

The starter culture of *Lactobacillus* spp. was obtained from the microbial stock culture in the laboratory of Bioprocess Technology, School of Industrial Technology, Universiti Sains Malaysia. The *Lactobacillus* spp., namely *Lacticaseibacillus paracasei*, *Lactiplantibacillus plantarum*, *L. acidophilus*, *Lacticaseibacillus rhamnosus*, and *Limosilactobacillus reuteri* were isolated from various food sources such as fermented rice (tapai) and milk products. Each strain was grown in *Lactobacillus*

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MRS broth (Himedia, Mumbai, India) and incubated at 37°C for 24 hr, reaching the following initial cell densities: *L. plantarum* (1.87×10^9 CFU/mL), *L. paracasei* (1.02×10^8 CFU/mL), *L. acidophilus* (2.17×10^9), *L. rhamnosus* (5.12×10^8 CFU/mL), and *L. reuteri* (3.02×10^8 CFU/mL). The strain was preserved at -20°C in MRS broth containing 50% (v/v) glycerol. Before fermentation, the strain was thawed from -20°C storage and cultured overnight in MRS broth at 37°C to prepare the inoculum.

Preparation of fruit juices

Commercially available fruit juice powders were obtained from BabyMommom, a local company. Five types of fruit juices were used: mango, pineapple, dragon fruit, cranberry, and mixed berry. The mixed berry powder was a blend of strawberry, blueberry, raspberry, and blackcurrant in equal proportions (1:1:1:1). Each juice powder was dissolved in distilled water at a 10% (w/v) concentration without pH adjustment. The prepared juices were then autoclaved at 115°C for 15 min before use.

Fermentation of fruit juices with *Lactobacillus* spp.

The *Lactobacillus* spp. overnight culture (1 mL) was inoculated into 10 mL of different sterile fruit juices; mango juice, pineapple juice, dragon fruit juice, cranberry juice, and mixed berry juice. The inoculated juices were incubated at 37°C for 72 hr, without shaking. All the fermentations were conducted in triplicates.

Analytical methods

Growth profile

The growth profile of *Lactobacillus spp.* on fermented fruit juices for 72 hr at different time intervals was determined. 200 microliters (µL) of the sample were added into a microplate and measured by HALO MPR-96 Visible Microplate Reader (Dynamica, Victoria, Australia) at an optical density (OD) of 595 nm.

Cell viability

Cell viability was determined by using the standard plate count method. Serial dilution (10^1- 10^9) of each fermented fruit juice was prepared with autoclaved distilled water. A series of tenfold serial dilutions (ranging from 10^1 to 10^9) was prepared for each fermented fruit juice sample. Autoclaved distilled water was used as the diluent to maintain sterile conditions and avoid contamination. The serial dilution process was initiated by adding 100 µL of the fermented fruit juice sample into 900 µL of autoclaved distilled water, resulting in an initial dilution factor of 10 (10^1 dilution).

Subsequent dilutions were prepared by transferring 100 µL of the previous dilution into a new tube containing 900 µL of autoclaved distilled water. This process was repeated sequentially to achieve final dilution factors ranging from 10^2 to 10^9. Each dilution was thoroughly mixed to ensure homogeneity before proceeding to the next step. 50 µL of diluted fruit juices (10^5 - 10^9) was streaked onto MRS agar medium and incubated at 37°C for 24 to 48 hr. The plate containing 100-300 colonies was measured. The colony (cell viability) will be counted and expressed as log colony-forming units per milliliter of the sample (log CFU/mL).

$$Cell \quad viability = log \quad CFU/ml$$

Specific growth rate

Time again *Lactobacillus* spp. growth profile was plotted during the logarithmic phase and the specific growth rate (Sg) was calculated using the following equation;

$$Sg \quad = ln \quad Fm \quad - ln \quad Im/t$$

Where: Fm is the amount of growth after t time (t) and Im is the amount of growth at the beginning time.

Lactic acid production

The determination method of lactic acid concentration was adapted from Borshchevskaya *et al*. (2016). A 0.2% iron (III) chloride solution was prepared at 25°C. In the assay, 100 µL of fermented fruit juice samples were centrifuged using a microcentrifuge (110 rpm) for 10 min. The supernatant (50 µL) was added to 2 mL of 0.2% solution of iron (III) chloride and vortexed. 200 µL of the solution was dispensed into the microplate and analyzed using the HALO MPR-96 Visible Microplate Reader at an absorbance of 405 nm. The concentration of lactic acid was determined using the following equation, derived from the lactic acid standard curve.

$$y = 0.0762x - 0.0109$$

pH

The pH of the fruit juices was measured by using a pH meter (Mettler-Toledo, Greifensee, Switzerland) before and after fermentation took place. The difference in pH before and after the fermentation process was evaluated.

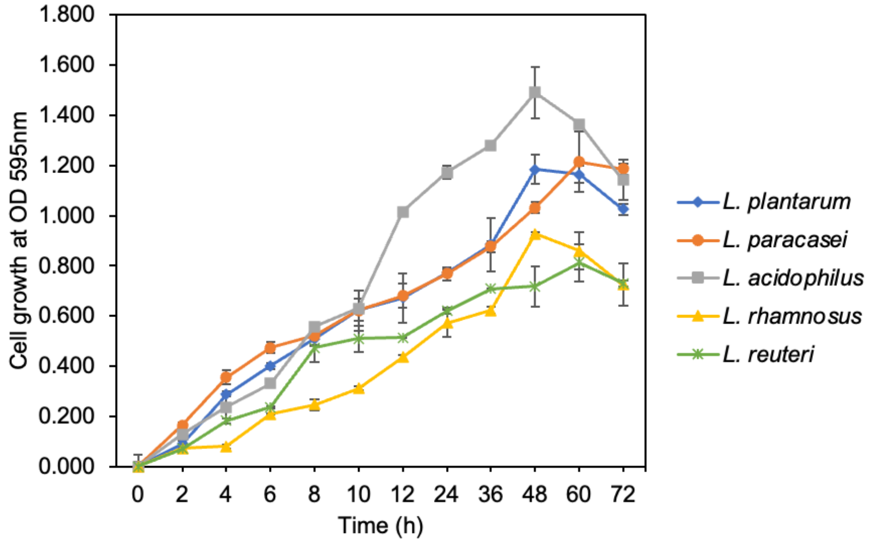
Statistical analysis

All data collected were expressed as mean and standard error mean. The results obtained were subjected to analysis of variance (ANOVA) by SPSS Version 28 as well as by Microsoft Excel. All results were expressed based on triplicate determinations, involving six technical replicates and three biological replicates. The regression coefficients in a confidence level above 95% were considered significant ($p<0.05$) and the data was analyzed by the Tukey test. The significant correlation between lactic acid productivity and pH was performed using correlation analysis.

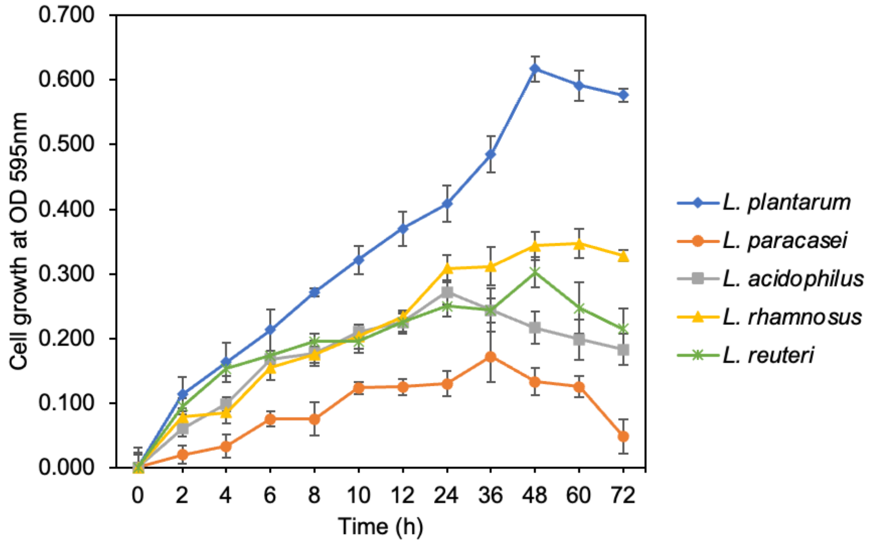
RESULTS AND DISCUSSION

Growth profile of *L. plantarum* fermented in fruit juices.

The growth of the *Lactobacillus* spp. fermented in the fruit juices was presented in Figure 1(a) to Figure 1(e).

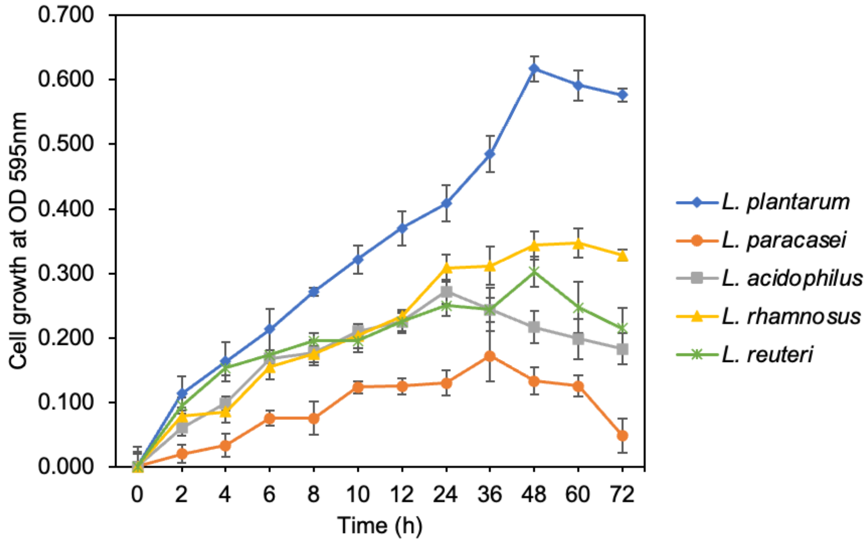


(a)



(b)

Fig. 1. The growth profile of Lactobacillus spp. fermented in five types of fruit juices. (a) refer to mango juice, (b) pineapple juice, (c) dragon fruit juice, (d) cranberry juice, and (e) mixed berry juice. The time intervals are 72 hr and measured at optical density (OD) of 595 nm. Values represent means of three determinations ± standard deviation.



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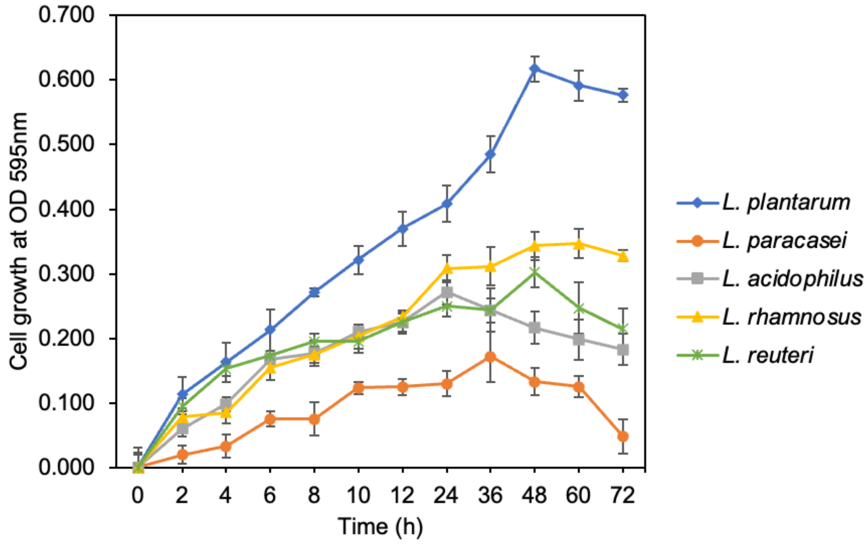
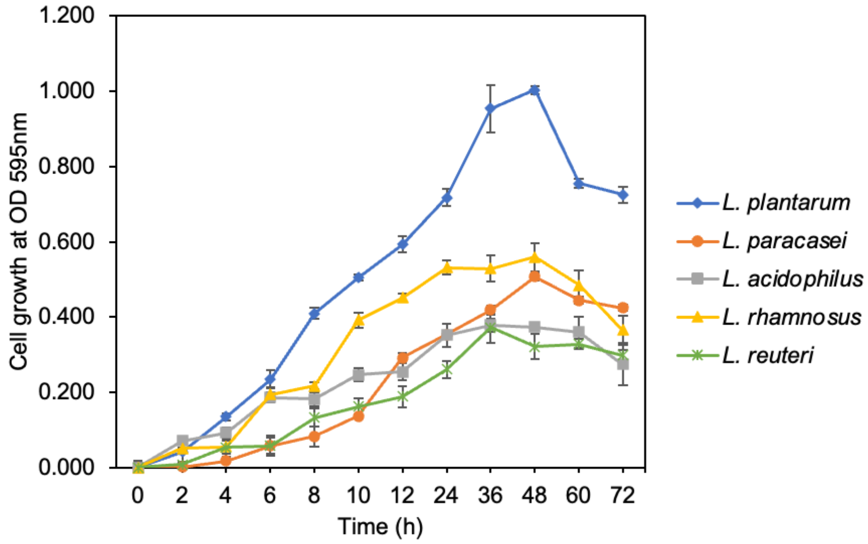


(d)



(e)

Fig. 1. (Continued) The growth profile of Lactobacillus spp. fermented in five types of fruit juices. (a) refer to mango juice, (b) pineapple juice, (c) dragon fruit juice, (d) cranberry juice, and (e) mixed berry juice. The time intervals are 72 hr and measured at optical density (OD) of 595 nm. Values represent means of three determinations ± standard deviation.

Figure 1(a) shows *L. acidophilus* has the highest cell count which is at 48 hr, with 1.490 ± 0.1007, compared to other *Lactobacillus* spp. in mango juice. Subsequently, the growth of *L. acidophilus* began to decline as its metabolic activity ceased. The second highest count was *L. paracasei* with 1.215 ± 0.1190 at 60 hr, followed by *L. plantarum* (1.185 ± 0.597) and *L. rhamnosus* (0.928 ±0.0049) at 48 hr respectively, while *L. reuteri* has the lowest bacteria count (4.415 ± 0.0746) even though at an early stage, it grew faster than *L. rhamnosus*.

In pineapple, dragon fruit, cranberry, and mixed berry juice (Figure 1b-e), *L. plantarum* consistently exhibited the highest cell count among the *Lactobacillus* spp. Its growth accelerated rapidly after the initial hr of fermentation. For instance, the maximum cell growth of *L. plantarum* fermented in pineapple juice was at 60 hr with 1.007 ± 0.0086 even though it had a slow start, while the maximum cell growth of *L. paracasei*, *L. acidophilus*, *L. rhamnosus* and *L. reuteri* were 0.487 ± 0.0137, 0.670 ± 0.0744, 0.767 ± 0.0101 and 0.335 ± 0.0131, respectively. A similar result can be seen during the fermentation of dragon fruit juice (Figure 1(c)) and mixed berry juice (Figure 1(e)) where *L. plantarum* has the maximum highest cell count (2.345 ± 0226 and 1.003 ±0.0114) compared to another *Lactobacillus* spp. On the other hand, in cranberry juice (Figure 1(d)), while *L. plantarum* reached its maximum growth at 48 hr with 0.617 ± 0.0189, other *Lactobacillus* spp. seems to be having a hard time growing, especially *L. paracasei*. It is almost as if they were not having any growth since the bacteria count was so small. This might be due to the acidic condition of cranberry juice (pH 2.94).

As reported by Soliman *et al*. (2015) and Vera-Peña and Rodriquez (2020), *L. plantarum* can tolerate acidic conditions (pH 2.0 & 3.0) and its optimal growth was at a pH of 6.0, while Di Biase *et al*. (2022) state that the minimum pH values estimated for *L. paracasei* strain ranged between pH 3.23 and pH 3.70. This shows that cranberry juice is not a good medium for *L. paracasei* to live and grow. The optimum pH for *L. acidophilus* was 5.5 - 6.0, *L. reuteri* was 5.5 and *L. rhamnosus* requires a maintained pH of 5.0 to increase the scale of biomass production (Polak-Berecka *et al*., 2011; Hinestroza-Córdoba *et al*., 2021; Gao *et al*., 2022). According to Śliżewska and Chlebicz-Wójcik (2020), the highest count of the *Lactobacillus* spp. was with a natural pH of 6.0. Given that the initial pH of all the fruit juices was below 6.0 (Table 2), particularly for cranberry and mixed berry juices which were highly acidic, the strains encountered difficulties in growth.

Lactobacillus plantarum is well-documented for its high acid resistance and ability to survive and grow in low-pH environments. A study by *Fidanza et al. (2021)* highlights that *L. plantarum s*trains demonstrate exceptional survival under acidic stress, making them suitable for acidic food products like fruit juices, with numerous studies showing that most strains exhibit varying degrees of acid tolerance through distinct mechanisms. Guo *et al.* (2017) report that *L. plantarum* exhibits strong potential to thrive under acidic stress conditions both *in vitro* and *in vivo*, making its acid tolerance a focal point of interest due to its wide-ranging applications in fermented foods and probiotic supplements. While the low pH of fruit juices poses a challenge to probiotic survival, it is hypothesized that incorporating lactic acid bacteria into such acidic environments may improve their resilience to subsequent acidic stress, such as that encountered in the gastrointestinal tract (Perricone *et al*., 2015).

Other than that, the availability of the nutrients in different fruit juices also may influence the growth of the strains. A study by Valero-Cases *et al*. (2017) states that the type of fruit juice used as a carrier notably affected the bacterium's growth. This statement aligns with a study by D'Amico *et al*. (2024) that the juice's chemical composition is important in ensuring the growth of bacterial strains. D'Amico *et al*. (2024) further reported that during fermentation, probiotic bacteria utilize the nutrients in the substrate, generating beneficial compounds like organic acids and bioactive metabolites. However, further work is necessary to determine the influence of the nutrient of fruit juices on the growth of different *Lactobacillus* species.

There are many factors affecting the growth of the lactobacillus species. Different probiotic species and strains exhibit completely different impacts, which makes it challenging to study a specific product because the efficiency of fermentation may be specific to some of these strains and species.

Cell viability and specific growth rate

The cell viability and the specific growth rate of *Lactobacillus* spp. were calculated after 24 hr of fermentation and presented in Table 1.

Cell viability

As shown in Table 1, the cell viability of most *Lactobacillus* spp. exceeded 10^6 CFU/mL, except *L. paracasei* in cranberry juice. For mango juice, *L. acidophilus* has the highest value which was 12.460 ± 0.172 log CFU/mL followed by *L. plantarum* (12.420 ± 0.0087 log CFU/mL), *L. rhamnosus* (12.280 ±0.275 log CFU/mL), *L. paracasei* (12.070 ± 0.036 log CFU/mL) and the lowest one was *L. reuteri* (11.800 ± 0.168 log CFU/mL). There was a significant difference (p<0.05) between *L. plantarum*, *L. acidophilus,* and *L. rhamnosus* with *L. reuteri*. However, *L. paracasei* showed no significant difference (p>0.05) with other *Lactobacillus* species.

In pineapple juice, the highest cell viability went to *L. plantarum* with 12.030 ± 0.653 log CFU/mL, while *L. reuteri* recorded the lowest value of cell viability with 10.420 ± 0.486 log CFU/mL. The viability of *L. paracasei* was 11.480 ± 0.500 log CFU/mL while *L. acidophilus* and *L. rhamnosus* shared the same value which was 11.640 log CFU/mL.

The same trend was shown in dragon fruit, cranberry, and mixed berry juices where *L. plantarum* exhibited the highest cell viability which was 12.330 ± 0.05 log CFU/mL, 11.770 ± 0.078 log CFU/mL and 12.410 ± 0.182 log CFU/mL, respectfully. While *L. reuteri* has the lowest value in dragon fruit juice (7.460 ± 0.085 log CFU/mL), *L. paracasei* showed the lowest value in cranberry juice which was 5.0 ± 0.006 log CFU/mL in terms of their viability. There was a significant difference (p<0.05) between *L. paracasei* in cranberry juice and other *Lactobacillus* spp. For mixed berry juice, *L. rhamnosus* has the lowest viability count which was 10.570 ± 0.369 log CFU/mL.

Table 1. The cell viability and specific growth rate of *Lactobacillus* spp. fermented in five types of fruit juices

Juices	*Lactobacillus* spp.	Cell viability (log CFU/mL)	Specific growth rate (h^{-1})
Mango	*L. paracasei*	12.07 ± 0.036^{ab}	0.079
	L. plantarum	12.42 ± 0.087^{a}	0.107
	L. acidophilus	12.46 ± 0.172^{a}	0.177
	L. rhamnosus	12.28 ± 0.275^{a}	0.053
	L. reuteri	11.8 ± 0.168^{b}	0.052
Pineapple	*L. paracasei*	11.48 ± 0.500^{ab}	0.103
	L. plantarum	12.03 ± 0.653^{a}	0.156
	L. acidophilus	11.64 ± 0.431^{a}	0.100
	L. rhamnosus	11.64 ± 0.163^{a}	0.153
	L. reuteri	10.42 ± 0.486^{b}	0.135
Dragon fruit	*L. paracasei*	12.29 ± 0.126^{a}	0.111
	L. plantarum	12.33 ± 0.050^{a}	0.170
	L. acidophilus	11.67 ± 0.277^{b}	0.151
	L. rhamnosus	11.30 ± 0.347^{b}	0.170
	L. reuteri	7.46 ± 0.085^{c}	0.127
Cranberry	*L. paracasei*	5.00 ± 0.006^{a}	0.101
	L. plantarum	11.77 ± 0.078^{b}	0.103
	L. acidophilus	11.58 ± 0.397^{b}	0.052
	L. rhamnosus	10.52 ± 0.454^{c}	0.069
	L. reuteri	10.88 ± 0.112^{c}	0.045
Mixed berry	*L. paracasei*	11.83 ± 0.534^{ab}	0.093
	L. plantarum	12.41 ± 0.182^{a}	0.186
	L. acidophilus	11.84 ± 0.424^{ab}	0.068
	L. rhamnosus	10.57 ± 0.369^{c}	0.156
	L. reuteri	11.35 ± 0.099^{bc}	0.064

Values denoted the means of three determinations ± standard deviation. Data with different alphabet superscript letters (a, b, c, and d) show significant differences at $p<0.05$, among different parameters for each juice, with multiple comparisons (one-way ANOVA, followed by Tukey's test). The cell counts at 24 hrs are used to calculate the specific growth rate of *Lactobacillus* spp. fermented in five types of fruit juices.

The assertion that *Lactobacillus* spp. can reach growth levels of 12 log CFU/mL when fermented in fruit juices like mango, pineapple, dragon fruit, and mixed berry juices might be because of the optimized fermentation condition, as well as the nutrient composition of fruit juices. Fermentation temperatures of around 37°C can optimize *Lactobacillus* activity, ensuring faster growth rates (Śliżewska & Chlebicz-Wójcik, 2020). Fruit juices contain sugars and essential vitamins that can serve as a rich nutrient source for *Lactobacillus* growth (Naseem *et al*., 2023). Some juices, particularly those high in fermentable sugars and favorable pH levels (such as mango) could promote rapid bacterial growth (Sourri *et al*., 2022). The availability of these nutrients can help explain the unusually high cell counts, though it might not be sufficient on its own.

Cell viability is a crucial metric in cell culture, used to correlate cell behavior with cell numbers, particularly in screening responses to drugs or chemical agents, and is defined as the number of live, healthy cells in a sample (Kamiloglu *et al*., 2020). As stated by the Food Safety and Quality Division (FSQD) as well as the Food Act 1983, Amendment of Regulation 26A, the probiotic cultures added shall remain viable and the viable count shall not be less than 10^6 CFU/mL or equal to 6 log CFU/mL. All of the *Lactobacillus* spp. have higher viable cell counts, by the regulations which are above 10^6 CFU/mL, except for *L. paracasei* which was inoculated into cranberry juice. Their viability was below 10^6 CFU/mL, which was only 5.0 ± 0.006 log CFU/mL. Thus, they did not comply with the regulation stated and cannot be used as a probiotic in this research, especially when added to the cranberry juice.

The viability of *L. paracasei* fermented in cranberry juice can be related to its growth as shown in Figure 1(d). We can see that the strain was having a hard time growing and living in the cranberry juice, thus making them not to be recommended as a probiotic agent in this research. The characteristics of the food matrix (acidity), the additional microorganisms, the level of oxygen in products, and their interactions are directly related to the viability of probiotics (Terpou *et al*., 2019). This statement aligns with a study by Meenu *et al*. (2024) that the survival of probiotic bacteria in fermented beverages is significantly affected by the specific strain utilized, alongside factors such as processing conditions, pH levels, and storage temperatures. The culture has to adapt and endure in fruit juices to deliver their advantages.

The viability of a probiotic is directly influenced by the properties of the probiotic involved, including strains and the quantity of inoculum used, as well as the food properties such as molecular oxygen, titratable acidity, the presence of sugar and salt, and the water activity as well as the pH where the stability of molecules, the activity of enzymes and the ultimately cellular metabolism are all directly impacted by pH levels (Maia *et al*., 2023). To support the growth, the various microorganism has a minimum, maximum, and optimum value of pH. According to Rengadu *et al*. (2021), nutrient depletion, low pH, and lactic acid buildup during storage can interfere with the survival of probiotic bacteria. These findings emphasize the importance of selecting appropriate probiotic strains and optimizing fermentation conditions to enhance viability and functionality.

Specific growth rate

The specific growth rate of *Lactobacillus* spp. fermented in different fruit juices was calculated during the exponential phase of the growth curve and presented in Table 1. According to the data, *L. acidophilus* exhibited the highest specific growth rate in mango juice at 0.177 h^{-1}, followed by *L. plantarum* at 0.107 h^{-1} and *L. paracasei* at 0.079 h^{-1}, with *L. reuteri* showing the lowest at 0.052 h^{-1}. In pineapple, cranberry, and mixed berry juices, *L. plantarum* demonstrated the highest specific growth rates of 0.156 h^{-1}, 0.103 h^{-1}, and 0.186 h^{-1}, respectively. In dragon fruit juice, both *L. plantarum* and *L. rhamnosus* exhibited the highest specific growth rate of 0.170 h^{-1}. The lowest specific growth rates were observed for *L. paracasei* in dragon fruit juices at 0.111 h^{-1}, respectively, and for *L. reuteri* in cranberry and mixed berry juices at 0.045 h^{-1} and 0.064 h^{-1}, respectively.

Filannino ***et al***. (2014) reported that the kinetic growth of *L. plantarum* fermented in pineapple juice for 24 hr was 0.15 ± 0.020 h^{-1}, whereas Mauro ***et al***. (2016) stated that the specific growth rate of *L. reuteri* fermented in the blueberry and carrot blend was 0.005 h-^{1}. Üçok and Sert (2020) reported that the maximum specific growth rate of *L. plantarum* was 0.551 h-^{1} after fermentation in MRS medium for 28 hr at 37°C, while in this study, the highest specific growth rate was observed in mixed berry juice at 0.186 h^{-1}. According to Śliżewska and Chlebicz-Wójcik (2020), fermentation medium can influence specific growth rates, which is the time for the bacteria to adapt to new media and not proliferate. The number of cells and the instantaneous velocity in a given interval time are related by the specific growth rate and it is predicted that the lower the specific value, the shorter the microorganism generation time where the environment, the growth condition, and the genetic affect the generation time (Mauro ***et al***., 2016). According to Jin and Kirk (2018) and Razmi ***et al***. (2023), pH is also a crucial environmental factor that regulates bacterial growth and activity, influencing microbial metabolism and specific growth rates by interfering with metabolic processes and altering bacterial physiological functions. The reduction of the maximum growth rate is one way to express the impact of acid toxicity (Yáñez ***et al***., 2008).

Lactic acid production of fermented fruit juices with different *Lactobacillus* spp. and the effect on the pH.

Figure 2 shows the lactic acid production during the fermentation of *Lactobacillus* spp. in fruit juices.

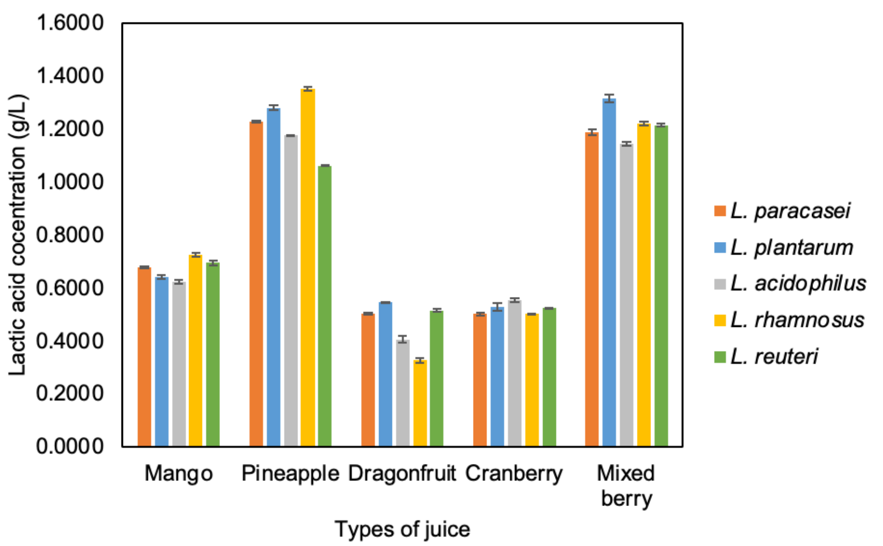


Fig. 2. Lactic acid produced by *Lactobacillus* spp. The concentration of lactic acid was calculated using an equation derived from the lactic acid standard curve (y = 0.0762x – 0.0109).

Based on Figure 2, *L. rhamnosus* produced the highest lactic acid concentration when it was fermented in mango (0.73 ± 0.0017 g/L) and pineapple (1.35 ± 0.0070 g/L), compared to other *Lactobacillus* spp (mango: *L. paracasei*: 0.68 ± 0.0046 g/L; *L. plantarum*: 0.64 ± 0.0110 g/L; *L. acidophilus*: 0.63 ± 0.0103 g/L; *L. reuteri*: 0.70 ± 0.0061g/L; pineapple: *L. paracasei*: 1.23 ± 0.0038 g/L; *L. plantarum*: 1.28 ± 0.0070 g/L; *L. acidophilus*: 1.18 ± 0.0015 g/L; *L. reuteri*: 1.06 ± 0.0020 g/L). However, for dragon fruit juice, the highest lactic acid concentration was produced by *L. plantarum*, which is 0.55 ± 0.0020 g/L while the other *Lactobacillus* spp.; *L. paracasei*, *L. acidophilus*, *L. rhamnosus,* and *L. reuteri*, each produced 0.50 ± 0.0045, 0.41 ± 0.0116 g/L, 0.33 ± 0.0065 and 0.52 ± 0.0050, respectively.

In cranberry juice, similar concentrations of lactic acid were produced across all five fermented juices with different *Lactobacillus* spp. added. Notably, *L. acidophilus* produced the highest lactic acid concentration at 0.56 ± 0.0074 g/L. In mixed berry juice, *L. plantarum* produced the highest lactic acid concentration at 1.32 ± 0.0190 g/L, while *L. paracasei*, *L. acidophilus*, *L. rhamnosus*, and *L. reuteri* produced 1.19 ± 0.0354 g/L, 1.15 ± 0.0060 g/L, 1.22 ± 0.0072 g/L, and 1.22 ± 0.0051 g/L, respectively. The initial lactic acid concentration, including the naturally occurring lactic acid in the fruit juices, is presented in the supplementary data (Figure 2.1). As shown in Figure 2.1, each fruit juice contains lactic acid naturally present before fermentation. This observation aligns with findings by Wang ***et al***. (2024), which demonstrated that raw juice inherently contains lactic acid, with its concentration increasing following the fermentation process.

Lactic acid production is widely recognized as a growth-associated process, where its accumulation during fermentation leads to a decrease in the pH (Popova-Krumova ***et al***., 2024). LAB is responsible for the production of lactic acid, which is the final product of the fermentation of carbohydrates (Abedi & Hashemi, 2020). Lactic acid is formed as a result of the consumption of glucose. Different LAB strains produce varying concentrations of lactic acid, which may explain the pH variation observed in this study (Degrain *et al*., 2020). Table 2 shows the changes in pH after fermentation took place.

Table 2. The pH of the fruit juices at 0 and after 24 hr of fermentation

Juice	Initial pH (0 hr)	pH of the juices after 24 hr				
		L. paracasei	*L. plantarum*	*L. acidophilus*	*L. rhamnosus*	*L. reuteri*
Mango	4.67 ± 0.198	3.13 ± 0.09^{8}a	3.20 ± 0.124^{b}	3.45 ± 0.095^{c}	3.34 ± 0.110^{d}	3.77 ± 0.09^{1}e
Pineapple	4.03 ± 0.203	3.00 ± 0.301^{a}	3.19 ± 0.237^{b}	3.36 ± 0.195^{c}	3.23 ± 0.113^{b}	3.64 ± 0.221^{d}
Dragon fruit	3.56 ± 0.086	2.94 ± 0.099^{a}	3.00 ± 0.146^{b}	3.42 ± 0.071^{c}	3.16 ± 0.121^{d}	3.50 ± 0.110^{e}
Cranberry	2.94 ± 0.205	2.84 ± 0.141^{a}	2.92 ± 0.098^{b}	2.90 ± 0.102^{ab}	2.75 ± 0.103^{c}	2.87 ± 0.200^{a}
Mixed berry	3.15 ± 0.084	2.87 ± 0.112^{a}	2.96 ± 0.160^{b}	3.10 ± 0.094^{c}	3.10 ± 0.104^{c}	3.09 ± 0.097^{c}

Values denoted the means of three determinations ± standard deviation. Data with different alphabet superscript letters (a, b, c, d & e) shows significant differences at p<0.05, among different parameters for each juice, with multiple comparisons (one-way ANOVA, followed by Tukey's test).

The initial pH is the pH of the fruit juices without the inoculation of *Lactobacillus* spp.

As shown in Table 2, the pH of all fruit juices decreased after inoculation with various *Lactobacillus* spp. during the fermentation process. The initial pH of the fruit juices was not adjusted to the optimum pH favorable for the strains, as the aim was to evaluate and select the best strain capable of thriving, growing, and producing the highest lactic acid under the natural conditions of these five juice types. After 24 hr of fermentation, the pH of the juices decreased across all treatments. Initially, the pH of the mango juice was 4.67. After 24 hr, fermentation with *Lactobacillus* strains led to a reduction in pH, with values ranging from 3.13 for *L. paracasei* to 3.77 for *L. reuteri*. This indicates a significant acidification of the mango juice, particularly with *L. paracasei* and *L. plantarum*, which caused the lowest pH values. Pineapple juice, with an initial pH of 4.03, also experienced a decrease in pH following fermentation. The pH values ranged from 3.00 with *L. paracasei* to 3.64 with *L. reuteri*. In dragon fruit juice, which started with a pH of 3.56, the pH dropped significantly after 24 hr of fermentation. The pH values ranged from 2.94 with *L. paracasei* to 3.70 with *L. reuteri*. This juice showed the most pronounced decrease in pH, particularly with *L. paracasei* and *L. plantarum*, indicating their strong fermentation capability. Cranberry juice had an initial pH of 2.94 and showed minimal changes in pH after fermentation, with values ranging from 2.75 with *L. rhamnosus* to 2.92 with *L. plantarum*. For mixed berry juice, which started with a pH of 3.15, the pH decreased to values ranging from 2.87 with *L. paracasei* to 3.10 with *L. acidophilus* and *L. rhamnosus*. This juice exhibited a moderate decrease in pH, with *L. paracasei* causing the lowest pH.

The production of lactic acid may cause the pH to become more acidic. According to Degrain *et al*. (2020), the pH of the vegetable decreased rapidly after 24 hr of fermentation, and during the entire fermentation for 72 hr, the inoculation of strain LAB consistently lowered the pH. In the same way, the fermentation of mango juice with different LAB strains resulted in a reduction of pH when compared with their unfermented mango juice (Cele *et al*., 2022). Dudek *et al*. (2024) state that the production of lactic acid increased as the pH decreased after the fermentation process.

However, the analysis revealed no significant correlation between lactic acid productivity and pH reduction in the fermented fruit juice (r=0.033, p>0.05). This suggests that pH reduction during fermentation may not be solely attributed to lactic production but could also be influenced by other factors such as the production of secondary organic acids, the variations in initial pH, buffering capacity of the fruit juice or the metabolic activities of the microorganisms beyond lactic acid synthesis, with the concentration of organic acids typically being strain-dependent (Liu *et al*., 2015; Bangar *et al*., 2022; Breidt & Skinner 2022; Jabłońska-Ryś *et al*., 2022; Chen *et al*., 2024). Popova-Krumova *et al*. (2024) reported that *L. plantarum* exhibited optimal growth and lactic acid production at an initial pH of 6.5. Similarly, Sarkar and Paul (2017) observed that the highest rate of lactic acid production by *Lactobacillus* spp. occurred at an initial pH of 6.5. Furthermore, Dudek *et al*. (2024) demonstrated that buffering the pH to 6.5 increased the lactic acid production by lactic acid bacteria. However, since the initial pH of the fruit juices was maintained in this study, this might have affected the productivity of lactic acid.

The production of organic acids, both in quantity and type, depends on the microorganism species, culture medium composition, and growth conditions, with *Lactobacillus* strains being particularly notable for their high acid resistance, efficient organic acid production, and overall productivity (Bangar *et al*., 2022). Peyer *et al*. (2017) and Breidt and Skinner (2022) highlight how the buffering capacity of fermentation media can influence pH changes, indicating that pH reduction is not solely dependent on lactic acid production and implying that pH reduction is influenced by multiple factors beyond lactic acid production.

The findings demonstrate that while lactic acid production plays a role in pH reduction during fermentation, it is not the sole factor influencing the observed changes in pH. Other variables, including the variations in initial pH, buffering capacity of fruit juices, and additional metabolic activities of microorganisms, may significantly contribute to pH stability. These results underscore the complexity of the fermentation process and the need for further investigation into the interplay between microbial metabolism and medium composition.

CONCLUSION

This study confirms that *Lactobacillus* spp. can successfully ferment fruit juices, with *L. plantarum* demonstrating the highest growth, lactic acid production, and cell viability. These findings highlight the potential for developing non-dairy probiotic beverages. Further research should focus on optimizing fermentation conditions and assessing the sensory and nutritional attributes of these beverages to enhance consumer acceptance.

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ETHICAL STATEMENT
Not applicable

CONFLICT OF INTEREST
The authors declare no conflict of interest.

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