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Development of a synbiotic longan beverage: a nutritious and functional blend

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Abstract

Synbiotic foods, which synergistically combine probiotics and prebiotics, have garnered attention for their potential health benefits. In line with this trend, this study aimed to develop a synbiotic beverage using longan fruit as the base and fructo-oligosaccharides (FOS) as the prebiotic source. Two strains of *Lactobacillus* probiotics, *Lactobacillus casei* 431 and *Lactobacillus plantarum* KM001, were used to ferment the longan juice with and without FOS. Total soluble solids, reducing sugar, pH, total acid production, and viable cell count of *Lactobacillus* microorganisms were evaluated to determine the optimal fermentation duration. The findings indicated that fermenting the juice for 12 hrs at 37°C aligned with the criteria established by the FAO/WHO for probiotic foods. Furthermore, compared to *L. casei* 431, the longan juice containing FOS fermented with *L. plantarum* KM001 showed a better survival rate in a simulated gastrointestinal environment. Additionally, the microorganism count in this beverage remained relatively constant throughout its 28-days shelf life at 4°C. These findings underscore the synbiotic longan beverage as a functional drink that combines viable probiotics with prebiotic FOS.

1. Introduction

Synbiotic beverages combine the benefits of probiotic microorganisms and prebiotic nutrients, offering consumers potential health advantages (Rodríguez *et al*., 2021). Probiotics are living microorganisms consumed in sufficient quantities to improve health (Hill *et al*., 2014). Prebiotics, such as galacto-oligosaccharides (GOS), inulin, and fructo-oligosaccharides (FOS), serve as nutrition for probiotics, supporting their growth and survival (Fonteles and Rodrigues, 2018). These prebiotics are resistant to digestion and are not absorbed in the gastrointestinal tract. Instead, they are utilized by the intestinal microflora (Mohanty *et al*., 2018). FOS are particularly effective as prebiotics because they can be readily utilized by probiotics without requiring modification by the human body. The recommended daily intake of FOS typically ranges from 1 to 4 g (Al-Sheraji *et al*., 2013; Nararatwanchai, 2021).

The most commonly used lactic acid bacteria (LAB) as probiotics in beverages are *Lactobacillus* and *Bifidobacteria* (Iqubal *et al*., 2021), including *L. acidophilus*, *L. johnsonii*, *L. rhamnosus*, *L. casei*, *L. plantarum*, *B. bifidum*, and *B. longum*. They have been shown to have significant benefits such as the extension of intestinal villi cells, inhibition of the *Trichinella spiralis* parasite, and inhibition of the *Plasmodium chabaudi* pathogen in rats (Chakkaravarthi and Aravind, 2021). *L. plantarum*, another popular microorganism found in beverages, contributes to increasing the shelf life and enhancing the flavor of fermented beverages. It has also been demonstrated to increase the nutritional benefits, including both the amount of phenolic compounds and antioxidant activity in fermented beverages (Plessas, 2022).

Longan (*Dimocarpus longan* Lour.), a highly esteemed subtropical fruit in Southeast Asia known as "dragon eye" due to its appearance, is commercially

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grown in countries like China, Vietnam, Australia, and Thailand (Zhang *et al*., 2020). The fruit's juicy and sweet pulp is rich in B vitamins, vitamin C, iron, magnesium, phosphorus, potassium, and other essential vitamins and minerals (Shahrajabian *et al*., 2019). It also contains phytochemicals such as flavonoids, alkaloids, and carotenoids, which possess medicinal properties that aid in blood circulation, support the nervous system, prevent memory loss, relieve insomnia, and alleviate body aches and pains (Zhang *et al*., 2020). Longan pulp, which has been used in traditional Chinese medicine for centuries (Zhang *et al*., 2020), is also ideal for developing synbiotic beverages. This is because it has a lot of polysaccharides, which stimulate the growth of intestinal microflora and have important biological effects like anti-diabetic and anti-viral activities, immune stimulation, and prebiotic properties (Huang *et al*., 2019; Rong *et al*., 2019). Consequently, polysaccharides from natural sources have attracted significant interest in pharmacology and biochemistry due to their potential therapeutic benefits.

This research primarily aimed to create a synbiotic beverage using fruits, specifically designed for individuals with dairy allergies. The longan fruit was selected owing to its large quantities during the harvest season, high nutritional value, and rich prebiotic content. The study investigated the use of longan in the development of a synbiotic beverage, delving into various aspects such as optimization of fermentation time, the impacts of prebiotic FOS and selected probiotic strains, enhancing probiotic survival in simulated gastrointestinal conditions, maintaining stability during storage, and evaluating the nutritional makeup of the longan-based synbiotic beverage. Ultimately, our goal was to offer a non-dairy beverage alternative enriched with longan-derived synbiotics.

2. Materials and methods

2.1 Longan juice preparation

The longan fruit variety "Daw" was obtained from a commercial orchard in Lamphun, Thailand. The fruit was washed, peeled, and cored to extract fresh pulp immediately after harvest. The extraction process involved using drinking water at a ratio of fresh longan pulp to drinking water of 3:1 (w/w). A juicing machine/blender (Philips Co., Ltd., Netherlands) was utilized to blend the mixture, and a two-layer filter cloth was employed to remove solids and obtain the longan juice. To enhance the nutritional value and provide synbiotic properties, fructo-oligosaccharides (FOS) (SU CHEM, Bangkok, Thailand) were added to the longan juice at a concentration of 30 g/L. The longan juice, with a total solid content of 13.48±0.12°Bx and a reducing sugar content of 48.49±0.41 g/L, as well as the longan juice with FOS, with a total solid content of 15.85±0.07°Bx and a reducing sugar content of 49.71±0.25 g/L, were both pasteurized at 63-65°C for 20 mins. Subsequently, they were cooled in an ice bath until reaching a temperature of 35-37°C before adding the starter culture.

2.2 Probiotic strains and starter culture preparation

For the synbiotic longan beverage, two strains of probiotics were used: *L. casei* 431 and *L. plantarum* KM001. The first strain, *L. casei* 431, was obtained in lyophilized form from Christian Hansen®, Hørsholm, Denmark. Another strain, *L. plantarum* KM001, was a very powerful probiotic strain that Associate Prof. Dr. Pinmanee Kwanmuang and her research group screened, identified, studied, and tested (unpublished data). *L. plantarum* KM001 was deposited at the culture collection of the School of Industrial Education and Technology, KMITL, Thailand.

To prepare the *L. casei* 431 starter culture, 25 mg of the lyophilized strain was mixed with 100 mL of pasteurized longan juice. The mixture was incubated under anaerobic and static conditions at 37°C for 8-10 hrs, or until a bacterial count of 8 log CFU/mL was achieved. Similarly, the *L. plantarum* KM001 starter culture was prepared by inoculating purified colonies of the strain on de Man Rogosa Sharpe (MRS; HIMEDIA, Mumbai, India) agar into 100 mL of pasteurized longan juice. The incubation conditions were the same as those for *L. casei* 431.

2.3 Preparation of synbiotic longan beverage and fermentation profile

Four distinct formulations of the synbiotic longan beverage were prepared as follows: formula i: *L. casei* 431 longan beverage, fermented longan juice using *L. casei* 431; formula ii: *L. casei* 431 longan-FOS beverage, fermented longan juice with FOS using *L. casei* 431; formula iii: *L. plantarum* KM001 longan beverage, fermented longan juice using *L. plantarum* KM001; and formula iv: *L. plantarum* KM001 longan-FOS beverage, fermented longan juice with FOS using *L. plantarum* KM001. The fermentation of these synbiotic longan beverages took place in 100-mL glass bottles, with each bottle containing 50 mL of longan juice, with or without FOS (as described in Section 2.1). The fermentation process began by inoculating 2.5 mL (5% v/v starter) of the probiotic starter culture with a cell density of 8 log CFU/mL (as detailed in Section 2.2) into 47.5 mL of each formula of pasteurized longan juice. The mixture was then incubated under anaerobic and static conditions at 37°C for 24 hrs.

Throughout the 24-hrs fermentation process, samples were collected every 3 hrs to monitor changes and determine the optimal fermentation time. The total soluble solids, reducing sugar, pH, total acid production (in the form of lactic acid), and viable cell count of *Lactobacillus* microorganisms were measured. Based on the results obtained, the optimal fermentation time for the synbiotic longan beverages was selected for further study.

2.4 Survival of probiotics assayed under simulated gastrointestinal conditions

The viability of the probiotics in the synbiotic longan beverage formulations i, ii, iii, and iv (see Section 2.3) with the optimal fermentation time was assessed by subjecting them to simulated gastric and intestinal conditions. To make the simulated gastric solution, 3 g/L of pepsin from porcine gastric mucosa (Sigma-Aldrich Co., St. Louis, USA) and 5 g/L of NaCl were mixed with a 1 M HCl solution to lower the pH to 2. For the simulated intestinal solution, 8 g/L of pancreatin from porcine pancreas (Sigma-Aldrich Co., St. Louis, USA), 36 g/L of oxgall (Neogen Crop., Lansing, USA), and 5 g/L of NaCl with the pH set to 8 with 1 M NaOH were used.

To begin the experiment, the synbiotic longan beverage samples were first centrifuged (Eppendorf Himac Technologies Co., Ltd., Tokyo, Japan) at 7370×*g*, 4°C for 10 mins. The resulting precipitate of 0.5 g of probiotic cells was then mixed with 5 mL of simulated gastric solution in a test tube and incubated at 37°C for 60 mins. The pH value was then adjusted to 7.0 with 1 M NaOH, and then 1.25 mL of simulated intestinal solution was added. The volume was then made up to 10 mL with phosphate-buffered saline (PBS) buffer pH 7.4, and the mixture was incubated at 37°C for 180 mins. The viable cell counts of the samples at the end of the gastric simulation and the end of the intestinal simulation were then measured to determine the effect of the simulated gastrointestinal conditions on the survival of probiotics.

2.5 Synbiotic longan beverage changes during storage

Each of the four beverage formulas (see Section 2.3) was stored in separate 100-mL glass bottles at 4°C for 28 days. Samples were collected every 7 days and analyzed for total soluble solids, reducing sugar, pH, total acid production, viable cell count, total phenolic compounds, and antioxidant activity.

2.6 Nutritional analysis of a selected synbiotic longan beverage

The nutritional analysis of the selected synbiotic longan beverage, which was enriched with FOS and fermented using *L. plantarum* KM001 at 37°C for 12 hrs, along with the nutritional value of longan juice, was conducted by the ALS Laboratory Group (Thailand) Co., Ltd., located in Bangkok, Thailand. The analysis included the following parameters: calories and carbohydrates, including dietary fiber, was assessed using the Method of Analysis for Nutrition Labeling (Sullivan and Carpenter, 1993); total dietary fiber was measured using the Association of Official Analytical Collaboration (AOAC) International method 985.29; ash content was determined using the AOAC International method 940.26A; protein content was analyzed using the AOAC International method 981.10; fat content was determined using the AOAC International method 922.06 (AOAC International, 2019). The total sugars, fructose, glucose, and sucrose were analyzed using the method described by Bugner and Feinberg (1992).

2.7 Analytical methods

The total soluble solids were measured in °Brix using a digital refractometer (Hanna Instrument Inc., Romania). Reducing sugar was determined using the DNS (3,5-dinitrosalicylic acid; Loba Chemie Pvt., Ltd., India) method (Miller, 1959), with glucose as the standard. pH was determined using a digital pH meter (Oakton Instrument, USA), while acidity was measured by titration against 0.1 N NaOH and expressed as lactic acid concentration. The viable cell count was obtained by serial dilution and the spread plate method on selective MRS agar, supplemented with 10 g/L calcium carbonate and incubated under anaerobic conditions at 37°C for 48 hrs. Total phenolic compounds were measured using Folin-Ciocalteu's phenol reagent (Sisco Research Laboratories Pvt., Ltd., India) and gallic acid (Sisco Research Laboratories Pvt., Ltd., Maharashtra, India) as a phenolic compound standard. Total phenolic compounds were expressed as milligram gallic acid equivalent per milliliter (mg GAE/mL) (Chaikham and Apichartsrangkoon, 2012), and the antioxidant activity was determined using the DPPH (2,2-diphenyl-1-picrylhydrazyl; Sisco Research Laboratories Pvt., Ltd., India) method (Thitiratsakul and Anprung, 2014).

2.8 Statistical analysis

The experiments were performed in triplicate, and the results were reported as mean values with a standard deviation (SD). The data were subjected to statistical analysis using a one-way Analysis of Variance (ANOVA) with a completely randomized design (CRD). To determine significant differences between means, Duncan's New Multiple Range Test (DMRT) was applied at a confidence level of 95% ($p \leq 0.05$). The statistical analysis was carried out using SPSS 16.0 software (SPSS Inc., USA).

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3. Results and discussion

3.1 Preparation of synbiotic longan beverage and fermentation profile

Longan juice with and without the prebiotic FOS was fermented with two probiotic strains of *Lactobacillus*: *L. casei* 431 and *L. plantarum* KM001. The fermentation profile of the beverage (Figure 1) was examined to determine the optimal fermentation duration. The activity of the microorganisms led to a slight reduction in total soluble solids and reducing sugar (Figures 1a and 1b), as they consumed sugars and nutrients for growth (Figure 1e) and acid production (Figure 1d), resulting in a decrease in pH (Figure 1c). The population of *Lactobacillus* cells increased as fermentation progressed (Figure 1e). To establish the optimal fermentation period, the total acid production, pH value, and *Lactobacillus* cell count were considered based on those reported by National Research Council (1992). An optimal total acid content of 4-8 g/L as lactic acid and a pH value between 4.0-4.5 were targeted. Furthermore, it was crucial to maintain *Lactobacillus* cell counts at a minimum of 10^7 CFU/mL to ensure compliance with probiotic food requirements, as highlighted by Garcia *et al.* (2020), and to ensure the desired synbiotic balance in the final product.

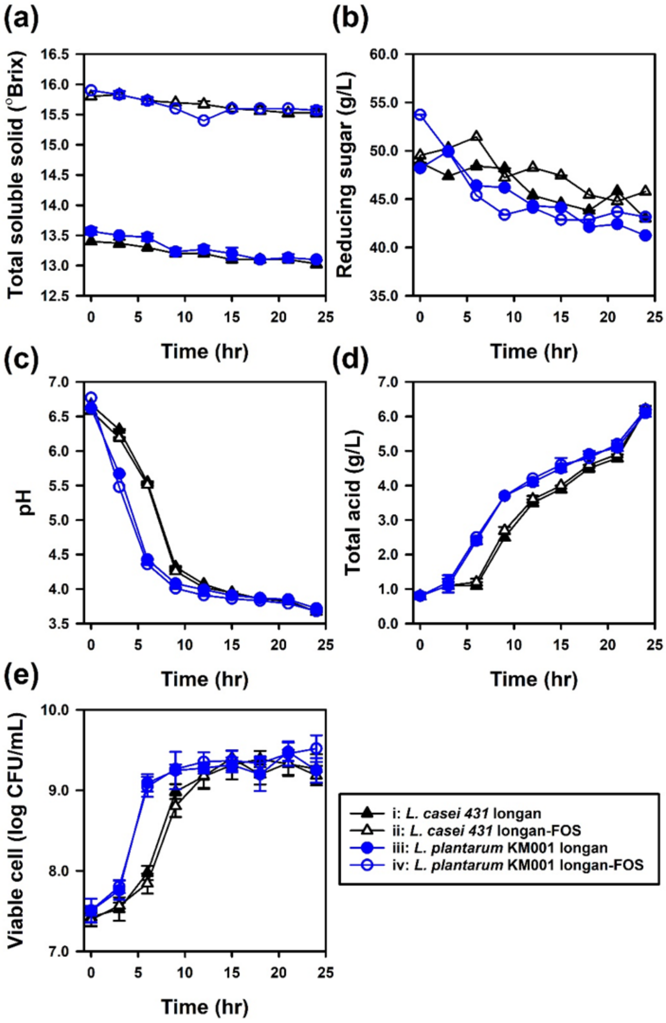

Figure 1. Changes during synbiotic longan juice fermentation, with or without FOS, using *L. casei* 431 and *L. plantarum* KM001.

When comparing the different formulas used, it was observed that longan juice without FOS had lower initial total soluble solids compared to those with FOS (Figure 1a). For formulas i and iii, fermented with *L. casei* 431 and *L. plantarum* KM001 respectively, the initial total soluble solids were 13.40 and 13.57°Bx. However, the addition of FOS led to higher initial total soluble solids, reaching 15.80 and 15.90°Bx for formulas ii and iv, fermented with *L. casei* 431 and *L. plantarum* KM001 respectively. A previous study by Kumar *et al.* (2020) has demonstrated that the presence of sugars and oligosaccharides in fruit juices stimulates the fermentative activity of lactic acid bacteria, resulting in acid formation and a decrease in pH. A similar finding has been reported for probiotic beverages made from other fruits, such as *Theobroma grandiflorum*, a tropical fruit native to the Brazilian Amazon, where pH decreased and lactic acid content increased during fermentation with *L. casei* (Pereira *et al.*, 2017). Lactic acid, the primary metabolite of lactic acid bacteria, not only contributes to the desired acidification of the beverage but also inhibits pathogenic and food spoilage microorganisms, making the probiotic beverage safer for consumption (Rathore *et al.*, 2012; Wang *et al.*, 2021). Additionally, lactic acid plays a role in influencing the sensory properties of the beverage as an important aromatic compound (Onyango *et al.*, 2000; Kedia *et al.*, 2007).

According to Huang *et al.* (2019), the polysaccharide prebiotics presented in longan pulp promoted the growth of *L. plantarum*, *L. bulgaricus*, and *L. fermentum*. This explained the similar fermentation profiles observed between longan juice with and without FOS (Figure 1). The addition of FOS to longan juice did not significantly enhance the growth of probiotics since the juice already contained polysaccharide prebiotics. The optimal fermentation period for synbiotic longan juice production was determined to be 9-12 hrs. During this period, the growth of *L. casei* 431 and *L. plantarum* KM001 reached the stationary phase at 12 and 9 hrs, respectively. Similar findings have been observed in other studies using probiotic bacteria in synbiotic drinks made from different sources. Nakkarach and Withayagiat (2018) observed the successful growth of *L. plantarum* TC24 in a synbiotic drink made from riceberry, a Thai rice variety. Additionally, Li *et al.* (2021) demonstrated the suitability of *L. plantarum* for fermenting probiotic fruit and vegetable juices, resulting in the development of specific flavors and favorable sensory characteristics. Several brands of probiotic juice drinks, such as GoodBelly, Probi-Bravo Friscus, and Healthy Life

Probiotic Juice (Dey, 2018), also utilize *L. plantarum* in their formulations.

To ensure that the synbiotic longan beverage met the criteria for a probiotic food, the production process followed one or more specific criteria related to pH, acidity, viable cell numbers, and sensory parameters (National Research Council, 1992; Garcia *et al*., 2020). It was essential to maintain a pH range of 4.0-4.5 and viable cell numbers of no less than 7 log CFU/mL (Kumar *et al*., 2020). After 12 hrs of fermentation at 37°C, the synbiotic longan beverage met these criteria, with pH values ranging from 3.91 to 4.07 and viable cell numbers ranging from 9.17 to 9.35 log CFU/mL. In addition, both strains reached the stationary phase during this period. Therefore, a fermentation time of 12 hrs was chosen for the production of the synbiotic longan beverage for further studies on survival under simulated human gastrointestinal conditions and changes during storage.

3.2 Survival of probiotic assays under simulated gastrointestinal conditions

The survival of probiotics in a simulated gastrointestinal environment was evaluated for four formulations of synbiotic longan beverage that underwent fermentation at 37°C for 12 hrs. Table 1 presents the results of the simulated gastrointestinal tolerance test. Initially, the cell count of *L. casei* 431 in the synbiotic longan beverage, with and without FOS, was 11.25 and 11.29 log CFU/mL, respectively. After passing through the simulated gastric system, the cell count (log CFU/mL) decreased by 33.69% and 31.98%, respectively. Similarly, the synbiotic longan beverage fermented with *L. plantarum* KM001 exhibited initial cell counts of 11.27 and 11.26 log CFU/mL, with and without FOS, respectively. Following passage through the simulated gastric system, the cell count (log CFU/mL) slightly decreased by 8.43% and 6.22%, respectively.

These results showed that *L. plantarum* KM001 was more resistant to the acidic and gastric conditions of the stomach than *L. casei* 431. The presence of hydrochloric acid and pepsin substantially decreased the probiotic cell count of *L. casei* 431 relative to *L. plantarum* KM001. However, adding FOS to both strains did not enhance their survival. According to Zhu *et al.* (2020), these simulated conditions closely resemble actual circumstances in which gastric digestion can destroy or damage *Lactobacillus* microbial cells.

After simulating the gastric system, a 180-mins assay was administered on the simulation of the intestinal system. The synbiotic longan beverage, whether fermented with *L. casei* 431 alone or with the addition of FOS, yielded viable cell counts of 8.65 and 8.95 log CFU/mL, respectively. When fermented with *L. plantarum* KM001, the counts were 9.79 log CFU/mL with FOS and 8.56 log CFU/mL without FOS. These findings suggested that *L. casei* 431 had better resilience in intestinal conditions, whereas *L. plantarum* KM001 was found a decline in cell viability. This could be due to the unique adaptive capabilities inherent to the *L. casei* 431 strain. Since the small intestine is more alkaline than the stomach, bacteria need adaptability. *L. casei* 431 might possess advanced pH-balancing abilities, which help it adjust and thrive in this environment. When observing the combined effects of the entire gastrointestinal system, FOS didn't impact *L. casei* 431's viability but did boost that of *L. plantarum* KM001. The most resilient beverage under conditions mirroring the human gastrointestinal system was the longan juice fermented with *L. plantarum* and enriched with FOS. Yuasa *et al*. (2021) found similar outcomes in their study of citrus juice fermentation using *L. plantarum* and *L. pentosus*, noting these probiotics maintained a significant cell count of 10.08 log CFU/mL, hinting at their potential gastrointestinal benefits.

Mohammadi *et al*. (2021) used *L. casei* and *L. plantarum* to develop a synbiotic functional drink with sprouted seeds of millet, rye, alfalfa, and prebiotics. Their research demonstrated that the addition of inulin and FOS as prebiotics enhanced the survival rate of probiotics under simulated human gastrointestinal conditions. The synbiotic drink contained 8 log CFU/mL of microorganisms with a 55% survival rate after being assessed in the simulated intestinal system. Similarly, Oliveira *et al*. (2011) found that prebiotics promote the growth and survival of probiotics in the human gastrointestinal tract.

Table 1. Viable cell counts of probiotics in the synbiotic longan beverage after the *in vitro* gastrointestinal tolerance test.

Synbiotic longan beverage formula*	Before treatment (log CFU/mL)	After gastric solution treatment (log CFU/mL)	After gastric and intestinal solution treatment (log CFU/mL)
i: *L. casei* 431 longan beverage	11.29±0.42	7.68±0.35	8.95±0.09
ii: *L. casei* 431 longan-FOS beverage	11.25±0.84	7.46±0.33	8.65±0.37
iii: *L. plantarum* KM001 longan beverage	11.26±0.63	10.56±0.02	8.56±0.67
iv: *L. plantarum* KM001 longan-FOS beverage	11.27±0.51	10.32±0.27	9.79±0.03

Note: *Please refer to Section 2.3 for the details of each synbiotic longan beverage formula.

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Furthermore, de Oliveira *et al*. (2021) investigated the changes in pH, total acid content, and total soluble solids when *L. rhamnosus*, *L. plantarum*, and *L. acidophilus* were used to ferment mango juice and carrot juice. They also examined the survival of probiotic bacteria under simulated gastrointestinal conditions. Their findings revealed that *L. plantarum* exhibited the highest survival rate, with over 7 log CFU/mL of surviving cells after testing in the simulated gastrointestinal conditions. Similarly, Abdul Manan *et al*. (2023) found that *L. plantarum* UALp-05 was the most tolerant under gastrointestinal tract conditions.

3.3 Changes in synbiotic longan beverage during storage

This part aimed to investigate the changes that occur during the storage of synbiotic longan beverages. After fermentation and subsequent storage at 4°C for 28 days, various parameters were analyzed, including total soluble solids, reducing sugar, pH, and total acid content (Table 2). The results indicated that all samples exhibited a decrease in reducing sugars and pH levels, while total soluble solids and total acid content increased. These findings aligned with previous studies that have reported the utilization of fructose by probiotics, resulting in acid formation and a decrease in pH (Peterson and Fred, 1920; Kumar *et al*., 2020).

The synbiotic longan beverage fermented with *L. casei* 431 showed a decline in viable cells from approximately 9.09-9.28 log CFU/mL on day 0 to around 7.73-7.91 log CFU/mL on day 28 of storage, but the content of lactic acid was increased from 3.35-3.53 g/L (day 0) to around 4.94-5.11 g/L on day 28 of storage (Table 2). The surviving bacteria result in increased acid production, while increased acidity can be toxic to cells. (Zhu *et al*., 2020). In contrast, the synbiotic longan beverage fermented with *L. plantarum* KM001 exhibited relatively stable microbial counts over the 28-day storage period, ranging from 9.02-9.21 log CFU/mL. It is worth noting that refrigeration is known to prolong the shelf life of probiotic products, although the number of viable cells can vary depending on the microbial strain (Mojikon *et al*., 2022).

Yoon *et al*. (2006) reported observations concerning the survival and stability of probiotic microorganisms, including *L. casei*, *L. delbrueckii*, and *L. plantarum*, during cold storage, specifically in the production of probiotic cabbage juice. Their study found that the number of *L. casei* declined dramatically over a 4-week storage period for the fermented cabbage juice. In contrast, the numbers of *L. plantarum* and *L. delbrueckii* remained, with a higher survival rate throughout the shelf life. These findings align with our study, where *L. plantarum* KM001 demonstrated a higher survival rate compared to *L. casei* 431 during a 28-day storage at 4°C. The greater survival rate of *L. plantarum* was attributed to its higher resistance to acid and lower pH compared to *L. casei*, as also discussed by Yoon *et al*. (2006).

During storage, the effect of prebiotics on the probiotic viability and acid content of synbiotic beverages seems to be strain-dependent. In our study, the addition of FOS had no significant effect on the change in viability and acidity over the course of 28 days of refrigerated storage. Although the increase in acidity was similar for both strains, the survival rate of *L. plantarum* KM001 was higher than that of *L. casei* 431; this indicates that *L. plantarum* KM001 is more acid-tolerant than *L. casei* 431. Battistini *et al.* (2018) found that the addition of prebiotics to a soymilk-based synbiotic beverage did not affect the total acid content or the population of specific probiotic strains during storage. However, the inclusion of prebiotics such as FOS in beverage products may benefit the digestive system by promoting the growth of beneficial microorganisms.

An important observation from this study, in addition to the changes in microbial viability and acid content, is the significant increase in total phenolic compounds and antioxidant activity in the synbiotic longan beverage during the 28-day storage period (Table 2). Phenolic compounds, a prominent category of antioxidants naturally present in many foods, play a crucial role in combating oxidative stress, which can subsequently prevent a myriad of chronic diseases. The elevation in total phenols during storage can likely be attributed to the enzymatic actions of the probiotic strains used. Specifically, enzymes such as cellulase and amylase, which are associated with probiotics, have been documented to facilitate the release of bioactive compounds from raw materials (Zhao *et al*., 2021). This surge in phenolic concentration signifies the potential health benefits that the beverage might offer beyond its probiotic properties.

Parallel to the rise in total phenols, the beverage also showcased an augmentation in its antioxidant activity over the storage period. Antioxidants are indispensable in neutralizing free radicals within the body, entities that can otherwise induce cellular damage. This pronounced antioxidant activity emphasizes the beverage's reinforced potential in mitigating oxidative stress, making it not only a probiotic supplement but also a protective agent against oxidative cellular impairments.

In corroboration with our findings, other research endeavors have also emphasized the health benefits of fermented beverages. For instance, fermented orange juice and sea buckthorn beverages, as documented in past studies, have manifested similar trends of increased

Table 2. The storage effect on the chemical compositions, viable cell count, and antioxidant property of synbiotic longan beverage during storage in the refrigerator (4°C).

Investigation factors	Storage time (days)	*L. casei* 431	*L. casei* 431 + FOS	*L. plantarum* KM001	*L. plantarum* KM001 + FOS
Total soluble solid (°Brix)	0	13.40±0.00^{c}	15.83±0.06^{c}	13.20±0.00^{c}	15.60±0.00^{c}
	7	13.53±0.06^{b}	15.90±0.00^{bc}	13.30±0.00^{b}	15.80±0.00^{b}
	14	13.53±0.06^{b}	15.97±0.06^{ab}	13.37±0.06^{a}	15.77±0.06^{b}
	21	13.73±0.06^{a}	15.93±0.06^{ab}	13.40±0.00^{a}	15.80±0.00^{b}
	28	13.77±0.06^{a}	16.00±0.00^{a}	13.43±0.06^{a}	15.93±0.06^{a}
Reducing sugar (g/L)	0	49.89±1.15^{a}	49.53±1.02^{a}	50.55±1.05^{a}	53.73±0.15^{a}
	7	49.44±0.00^{a}	49.22±0.15^{a}	49.11±0.15^{b}	54.21±2.08^{a}
	14	48.78±0.01^{b}	48.27±0.00^{bc}	47.23±0.94^{c}	46.78±0.00^{b}
	21	47.78±0.15^{b}	48.67±1.09^{b}	47.89±1.25^{c}	46.34±4.39^{b}
	28	47.23±2.19^{b}	47.28±0.00^{c}	47.45±3.44^{c}	45.56±1.41^{c}
pH	0	4.07±0.01^{a}	4.03±0.01^{a}	3.96±0.01^{a}	3.96±0.01^{a}
	7	4.03±0.01^{b}	4.02±0.01^{a}	3.94±0.01^{b}	3.90±0.00^{b}
	14	3.96±0.01^{c}	3.94±0.01^{b}	3.88±0.01^{c}	3.87±0.01^{c}
	21	3.82±0.01^{d}	3.82±0.01^{c}	3.80±0.01^{d}	3.80±0.01^{d}
	28	3.81±0.01^{d}	3.79±0.01^{c}	3.76±0.01^{e}	3.74±0.01^{e}
Total acid (g/L)	0	3.35±0.00^{e}	3.53±0.00^{d}	3.97±0.09^{c}	4.06±0.00^{d}
	7	3.70±0.00^{d}	3.70±0.02^{c}	4.23±0.35^{c}	4.41±0.00^{c}
	14	4.41±0.00^{c}	4.23±0.00^{b}	4.76±0.18^{b}	4.67±0.09^{b}
	21	5.03±0.09^{b}	4.94±0.00^{a}	5.03±0.09^{b}	4.85±0.09^{b}
	28	5.11±0.00^{a}	4.94±0.00^{a}	5.56±0.09^{a}	5.64±0.18^{a}
Viable cell count (log CFU/mL)	0	9.28±0.46^{a}	9.09±0.16^{a}	9.21±0.31^{a}	9.15±0.46^{a}
	7	8.93±0.41^{a}	8.53±0.50^{ab}	9.08±0.19^{a}	9.04±0.76^{a}
	14	8.86±0.38^{a}	8.96±0.53^{a}	9.17±0.77^{a}	9.02±0.59^{a}
	21	8.40±0.34^{a}	8.32±0.91^{ab}	9.16±0.65^{a}	9.04±0.50^{a}
	28	7.91±0.10^{b}	7.73±0.45^{b}	9.21±0.27^{a}	9.09±0.65^{a}
Total phenols (mg GAE/mL)	0	10.30±0.00	10.27±0.00	10.36±0.00	10.70±0.00
	28	13.00±0.00	12.22±0.00	13.13±0.00	12.76±0.00
Antioxidants (% inhibition)	0	17.48±0.00	17.55±0.00	16.69±0.01	17.27±0.01
	28	33.67±0.00	30.75±0.01	21.16±0.00	22.59±0.00

Values are presented as mean±SD. Values with different superscripts within the same column of each analysis are statistically significantly different at $p \leq 0.05$ using Duncan's Multiple Range Test. GAE: gallic acid equivalent.

phenolic compounds and antioxidant activities (Miranda *et al*., 2019; Tkacz *et al*., 2020). Such parallels further accentuate the health-promoting prospects of fermented drinks.

3.4 Nutritional analysis of selected synbiotic longan beverage

The nutritional analysis presented in Table 3 revealed that the selected synbiotic longan beverage has a higher energy and carbohydrate content compared to fresh longan juice. This disparity can be attributed to the addition of FOS in the synbiotic beverage. FOS, classified as fructan group carbohydrates and functional fibers, act as prebiotics that supports the growth and survival of probiotics, providing various benefits to the body (Slavin, 2013; Mohanty *et al*., 2018).

In contrast to regular carbohydrates, FOS stimulate the production of short-chain fatty acids (SCFAs), such as propionate, butyrate, and acetate, by intestinal microorganisms (Sabater-Molina *et al*., 2009). These SCFAs play a crucial role in intestinal system functioning, enhance mineral absorption, regulate fat and glucose digestion, and reduce the risk of colon cancer. Moreover, prebiotics like FOS promote the growth of beneficial microorganisms in the gut, thereby reducing the presence of harmful pathogens (Mohanty *et al*., 2018; Dávila *et al*., 2019).

According to Sabater-Molina *et al*. (2009), FOS provide 1.5-2.0 kcal of energy per gram, slightly

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Table 3. Nutritional value per 100 mL of fresh longan juice or synbiotic longan beverage fermented in longan juice with FOS using *L. plantarum* KM001.

Type of compositions	Fresh longan juice	Synbiotic longan beverage
Calories (kcal)	51.83	56.80
Carbohydrate (Include dietary fiber) (g)	12.30	13.50
Dietary fiber (Total) (g)	0.12	0.06
Ash (g)	0.50	0.35
Protein (g)	0.66	0.69
Fat (g)	0.00	0.00
Total sugars (g)	13.07	9.16
Fructose (g)	2.72	2.51
Glucose (g)	2.77	2.46
Sucrose (g)	7.58	4.19

increasing the overall energy content of the synbiotic longan beverage compared to unfermented longan juice. However, consuming the synbiotic longan beverage offers numerous advantages. It combines the nutritional benefits of longan as the primary ingredient, the prebiotic properties of FOS, and the probiotic effects of *L. plantarum* KM001. Non-dairy synbiotic beverages are gaining popularity among health-conscious consumers due to the increasing demand for natural and healthy food products as well as the growing prevalence of dairy allergies (Dahiya and Nigam, 2022; Yadav *et al*., 2022).

Fruit juices, such as longan juice, serve as excellent raw materials for probiotic fermentation as they contain sugars, vitamins, minerals, and phytochemicals that create an ideal environment for the growth of probiotic microorganisms. This fermentation process enhances the functional properties and nutritional value of beverages, ultimately promoting consumer health (Aspri *et al*., 2020; Valero-Cases *et al*., 2020).

As functional beverages, synbiotic drinks, when consumed regularly, provide positive impacts on the intestinal system, reduce the risk of various diseases, lower cardiovascular disease risk factors, and prevent metabolic syndrome and insulin resistance in the elderly (Mohanty *et al*., 2018; Cicero *et al*., 2021). Therefore, the synbiotic longan beverage represents an ideal alternative for health-conscious consumers seeking a functional beverage that offers both nutritional benefits and the positive effects of probiotics.

4. Conclusion

The investigation of a synbiotic longan beverage formulation revealed that *Lactobacillus plantarum* KM001 was superior to *Lactobacillus casei* 431 as a probiotic strain. It demonstrated impressive growth potential, robust survival in simulated gastrointestinal conditions, and a notable survival rate throughout a 28-day shelf life at 4°C. The presence of the prebiotic fructo-oligosaccharides (FOS) had no significant effects on the probiotic's growth or survival during storage but actually enhanced its survival in a simulated gastrointestinal test. Subsequently, the beverage fermented with *L. plantarum* KM001 and FOS emerged as the optimal formulation. Key metrics for this beverage, such as pH, total acidity, and cell viability, were recorded at 3.96, 4.06 g/L, and 9.15 log CFU/mL, respectively, satisfying probiotic food recommendations. Notably, this formulation showed the highest probiotic survival in the gastrointestinal simulation and maintained microbial stability over a 28-day storage period at 4°C, consistently achieving a survival rate above 9 log CFU/mL. Further benefits of this synbiotic longan beverage included an elevation in phenolic compound levels and antioxidant activity during storage. In summary, this study substantiates the potential of synbiotic longan beverage as a health-enhancing, nutritious, and functionally beneficial beverage.

Conflict of interest

The authors declare no conflict of interest.

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