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Effect of thermal treatments on the storage stability of *Lactobacillus acidophilus* La-14 tamarind juice with or without beta-glucans

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ABSTRACT

Aims: This study aimed to evaluate the effect of thermal treatment on the storage stability of *Lactobacillus acidophilus* La-14 tamarind juice with or without beta-glucans.
Methodology and results: *Lactobacillus acidophilus* incorporated with 6% (w/v) beta-glucans displayed the highest viability (17.28 $\log_{10}$ CFU/mL) as compared to other beta-glucans concentrations (0–8% w/v). The *L. acidophilus* with or without beta-glucans survived more than 80% after 5 h of sequential digestion. Tamarind juice was subjected to different thermal treatments (76 °C for 30 s or 90 °C for 60 s) and incorporated with *L. acidophilus* with or without beta-glucans. *Lactobacillus acidophilus* in tamarind juice without thermal treatment showed the highest viability (8.69 $\log_{10}$ CFU/mL), followed by thermal treatment at 76 °C for 30 s (>7 $\log_{10}$ CFU/mL), and thermal treatment at 90 °C for 60 s showed the lowest viability (>4 $\log_{10}$ CFU/mL), after 21 days at 4 °C. The pH, titratable acidity and viscosity of all *L. acidophilus*-tamarind juices demonstrated no changes throughout 21 days at 4 °C. Furthermore, thermal-treated tamarind juice (90 °C for 60 s) incorporated with *L. acidophilus* displayed the least change in total soluble solids (1.99 °Brix), while thermal-treated tamarind juice (90 °C for 60 s) with *L. acidophilus* and beta-glucans had the lowest color change (ΔE = 4.46), after 21 days of storage at 4 °C.
Conclusion, significance and impact of study: Thermal treatments (90 °C for 60 s) had contributed to the stability of *L. acidophilus*-tamarind juice with beta-glucans over 21 days of cold storage. This study shows thermal treated tamarind juice with *L. acidophilus* and beta-glucans is a potential functional non-dairy beverage catered for lactose intolerance individuals.

Keywords: Probiotic, prebiotic, functional beverage, storage stability, sequential digestion

INTRODUCTION

Probiotics are live microbiomes that can inhabit under intestinal environment and exert beneficial effect on human health (Shi *et al.*, 2016). According to Lomer *et al.* (2008), there are nearly three quarters of the total population suffers from lactose maldigestion. Owing to the fact of the high prevalence of lactose intolerance worldwide, non-dairy lactose-free probiotics product hereby plays a pronounced role in the food industry (Champagne *et al.*, 2018; Suri *et al.*, 2019).

The well-known functions of probiotics are lowering cholesterol levels, preventing antibiotic-related diarrhea and alleviating symptoms caused by lactose intolerance (Ataie-Jafari *et al.*, 2009; Fox *et al.*, 2015; Gayathri and Vasadha, 2018). Over the past decades, researchers focused on the functionality and application of probiotics from *Lactobacillus* and *Bifidobacterium* genus (Fenster *et al.*, 2019). *Lactobacillus acidophilus* La-14 is a Gram-positive, facultative anaerobic, non-spore-forming and rod-like microorganisms (Goldstein *et al.*, 2015) that plays an important role in protecting female health (Jang *et al.*, 2017). *L. acidophilus* La-14 possesses excellent characteristics such as high tolerance towards the harsh gastrointestinal environment, strongly adhesive to the intestinal epithelium, degrading oxalate, exert antimicrobial properties against pathogens and modulating the human immune system (Abdrabou *et al.*, 2018).

Although numerous reports had shown that probiotics

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food supplements could demonstrate beneficial action in the gastrointestinal tract, combining probiotics with prebiotics can further improve their health benefits towards consumers (Mohanty *et al.*, 2018). Prebiotics are defined as non-digestible food components which can selectively facilitate the growth of one or more probiotics, therefore promoting the host's biological functions (Gibson *et al.*, 2017). It can be embedded in a wide range of foods and has rapidly drawn a lot of interest from researchers due to its high nutritional and economic value (Al-Sheraji *et al.*, 2013; Padma Ishwarya and Prabhasankar, 2014; Konar *et al.*, 2016). Beta-glucans are non-digestible dietary fiber that is found in the cell walls of microorganisms, algae and plants such as oats and barleys (Cosola *et al.*, 2017). Due to its health-promoting properties, beta-glucans were explored as a potential prebiotic to protect probiotics through harsh environments such as acid, bile and storage through functional foods (Stack *et al.*, 2010).

Emerging non-dairy probiotics food products such as probiotics nutrition bars, breakfast cereal, fruits and vegetable-based juices, and snacks have acquired a high acceptance among consumers (Granato *et al.*, 2010; Ng *et al.*, 2019; Yee *et al.*, 2019; Lai *et al.*, 2020; Lai *et al.*, 2020). Moreover, recent studies had suggested that fruit juices are low-fat, alcohol-free, non-dairy and nutritious beverages containing abundant natural beneficial components such as minerals, vitamins, dietary fibers and antioxidants. This allows fruit juices to be an ideal carrier for probiotics (Pereira *et al.*, 2011; Ephrem *et al.*, 2018). Tamarind pulp is rich in vitamins A, B, C and organic acids (tartaric acid, citric acid and malic acid), hence demonstrating a high antioxidant potentiality (Rai *et al.*, 2018). However, tamarind contains polyphenol oxidase enzyme that could contribute to color changes upon storage (Obulesu and Bhattacharya, 2011). Nevertheless, methods such as thermal treatment could denatured the enzymes to prevent color changes (Moon *et al.*, 2020). Furthermore, it has been reported that thermal treatment could also maintain physicochemical properties in fruit juices by inactivating undesired microorganisms (Santhirasegaram *et al.*, 2013).

Probiotics are often incorporated into dairy products that may not be suitable for lactose intolerance individuals. Hence, lactose-free fruit juices such as tamarind juice could be an alternative carrier for probiotics. In order to prevent color change and maintain physicochemical properties of the probiotic-tamarind juice during storage, different thermal treatments had been explored in this study. To date, there is yet to be any research on incorporating thermal-treated tamarind juice with probiotics and beta-glucans. Hence, this study aimed to determine the suitability of thermal-treated tamarind juice as an ideal culture medium for probiotic *L. acidophilus* growth. In addition, the prebiotic effect of beta-glucans on *L. acidophilus* La-14 through simulated gastrointestinal conditions and storage in tamarind juice was evaluated. Moreover, the physicochemical properties of the *L. acidophilus*-tamarind juice were also assessed in this study.

MATERIALS AND METHODS

Culture preparation

Lactobacillus acidophilus La-14 was prepared according to Luciano *et al.* (2018) with some modification. Briefly, 0.5 g of *L. acidophilus* La-14 powder (Pure Encapsulations, USA) was cultivated in 100 mL of sterile Man, Rogosa, Sharpe (MRS) broth (Hi-Media, India) and incubated at 37 °C for 24 h. The cells were then washed twice with phosphate-buffered saline (PBS) through centrifugation (5840 R, Eppendorf, Germany) with a speed of 4500× *g* for 15 min at 4 °C. The collected cell pellets were suspended in 100 mL of sterile PBS and homogenized using a vortex mixer (VTX-3000 L, LMS, Japan). The cell counts were standardized by optical density measurement at 600 nm using a spectrophotometer.

Optimization of beta-glucans concentration

Food grade beta-glucans tablets (Solgar, USA) were manually ground with a mortar and a pestle into a fine powder. The beta-glucans solution was prepared at different concentrations (0, 2, 3, 4, 5, 6, 7 and 8% w/v) by dissolving beta-glucans powder into 100 mL of MRS broth (Hi-Media, India) and autoclaved at 121 °C for 15 min (HV-110, Hirayama, Japan). After cooling down the beta-glucans mixture to ambient temperature, 1% (w/v) of *L. acidophilus* La-14 culture was inoculated into the mixture and incubated at 37 °C for 24 h (Chaikham *et al.*, 2012). The viability of *L. acidophilus* La-14 in beta-glucans solution was carried out using the drop plate method by adding 10 μL of aliquots onto MRS agar. The plates were incubated at 37 °C for 48 h and the viable cell counts of *L. acidophilus* La-14 were calculated using Equation 1 and expressed as $\log_{10}$ CFU/mL (Phuapaiboon *et al.*, 2013). The optimal beta-glucans concentration was chosen based on the viability of *L. acidophilus* La-14 in MRS broth.

Colony forming units (CFU/mL) = Number of colonies formed / [Dilution factor × Volume plated (mL)] (1)

Preparation of simulated gastrointestinal juices

The preparation of simulated gastrointestinal juices was according to Chia *et al.* (2015) with modification. The simulated gastric juice (SGJ) was prepared by adding 3.5 mL of hydrochloric acid (Merck KGaA, Germany) and 1 g of sodium chloride (R&M Chemicals, UK) in 500 mL of distilled water. The pH of simulated gastric juice was adjusted to 2.0 using a pH meter (Eutech, USA) and sterilized at 121 °C for 15 min. After the solution was cooled down to room temperature, 1.6 g of pepsin (HmbG, Germany) was added into the mixture. On the other hand, simulated intestinal juice (SIJ) was prepared by adding 3.4 g of potassium dihydrogen phosphate (Midlothian, UK) into 95 mL of 0.1 M sodium hydroxide (Merck KGaA, Germany) and 125 mL of distilled water.

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The mixture was then topped up to 500 mL with distilled water and adjusted to pH 7.5 prior to sterilizing at 121 °C for 15 min. The bile salt (R&M Chemical, UK) (3 g) was added into the mixture after it cooled down to room temperature.

Sequential digestion

Sequential digestion was carried out according to Chia *et al.* (2015) with modification. One milliliter of *L. acidophilus* La-14 with beta-glucans (6% w/v) and without beta-glucans was added into 9 mL of sterile SGJ solution, respectively. The mixture was constantly agitated at 100 rpm in the water bath (Memmert, Germany) at 37 °C for 2 h. After 2 h, the mixture was washed with PBS by centrifuging at 3200× *g* for 15 min at 4 °C to remove the SGJ solution. The cell pellets were then transferred to SIJ solution for another 3 h of incubation. Similarly, the mixture was also washed with PBS by centrifuging at 3200× *g* for 15 min at 4 °C to remove the SIJ solution. The viability of *L. acidophilus* La-14 with or without beta-glucans was carried out using the drop plate method at each hour of SGJ and SIJ incubation. Furthermore, the viable cell counts of *L. acidophilus* La-14 with or without beta-glucans at 0 h of sequential digestion was served as control. The viability was calculated using Equation 1 and expressed as $\log_{10}$ CFU/mL. On the other hand, the survivability of *L. acidophilus* La-14 with or without beta-glucans was calculated using Equation 2 where N is the number of viable counts of cells released from microbeads at different times, and N_0 is the number of viable counts at 0 h.

$$\text{Survivability (\%)} = (\text{Log } N / \text{Log } N_0) \times 100\% \quad (2)$$

Preparation and storage stability of *L. acidophilus* La-14 in tamarind juice

The preparation of tamarind juice was in accordance with Tril *et al.* (2014) with modification. Fully ripened sour tamarind (*Tamarindus indica* LINN) was purchased from the local market. The tamarind juice was obtained by submerging 75 g of seedless tamarind pulp in 1500 mL of deionized water and blended using a juicer. The homogenized tamarind juice was then sieved three times to remove the debris. The supernatant part of the tamarind juice was collected (pH 3.6) and stored in sterile centrifuge tubes with parafilm under 4 °C prior to thermal processing. Different thermal treatments at different temperatures (76 °C and 90 °C) and duration (30 s and 90 s) were used on the tamarind juice samples prior to the incorporation of 1% (w/v) *L. acidophilus* La-14 with or without 6% (w/v) beta-glucans according to Sheehan *et al.* (2007) (Table 1). All the batches of tamarind juice treated with different thermal conditions and incorporated with *L. acidophilus* with or without beta-glucans (S1, S2, S3, S4 and S5) were stored at 4 °C for 21 days.

Viability and physicochemical properties of *L. acidophilus* La-14 in tamarind juice during storage

The viability of *L. acidophilus* La-14 in tamarind juice was determined on day 0, 7, 14 and 21 of storage at 4 °C using the drop plate method with MRS agar. The agar plates were incubated at 37 °C for 48 h. The viable cell counts were calculated using Equation 1 and expressed as $\log_{10}$ CFU/mL.

The pH, titratable acidity, total soluble solids, viscosity and color changes of *L. acidophilus* La-14 tamarind juice were evaluated on day 0, 7, 14 and 21 of storage at 4 °C. The pH was determined using a digital pH meter (pH 700, Eutech Instrument, USA). The titratable acidity was conducted according to Oladipo *et al.* (2014) where phenolphthalein was added into *L. acidophilus* La-14 tamarind juice as an indicator and titrated with 0.1 N sodium hydroxide to a pink endpoint. The titratable acidity was expressed as % lactic acid. Furthermore, the total soluble solids were measured using a refractometer (Bellingham Stanley, UK) and expressed as °Brix. Moreover, the viscosity of *L. acidophilus* La-14 tamarind juice was determined using a viscometer with spindle LV-1 at 100 rpm for 30 s (Brookfield Viscometer DV-II+ Pro, USA) and reported in millipascal-second (mPa·s). Lastly, the total color change (ΔE) was carried out using a colorimeter (ColorFlex EZ Spectrophotometer, Hunter Colorimeter, USA) where L* (intensity of black and white), a* (intensity of red and green) and b* (intensity of yellow and blue) were read by the reflectance from the instrument. The total color change was calculated using Equation 3.

$$\text{Total color change } (\Delta E) = \sqrt{\Delta a^{*2} + \Delta b^{*2} + \Delta C^{*2}} \quad (3)$$

Table 1: Tamarind juice treated with different thermal conditions and incorporated with *L. acidophilus* La-14 with or without beta-glucans.

Tamarind juice samples	Thermal treatments		Probiotic with or without prebiotic
	Temperature (°C)	Duration (s)	
S1	-	-	1% (w/v) *L. acidophilus* + 6% (w/v) beta-glucans
S2	76	30	1% (w/v) *L. acidophilus*
S3	76	30	1% (w/v) *L. acidophilus* + 6% (w/v) beta-glucans
S4	90	60	1% (w/v) *L. acidophilus*
S5	90	60	1% (w/v) *L. acidophilus* + 6% (w/v) beta-glucans

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Statistical analysis

All data were analyzed using Minitab Statistical Analysis (version 16, Minitab Inc., USA) and reported as mean ± standard deviation. One-way analysis of variance (ANOVA) and Tukey's post hoc test was used to evaluate the significant difference between means, where $p \leq 0.05$ was considered as significant.

RESULTS AND DISCUSSION

Optimization of beta-glucans concentration on the growth of *L. acidophilus* La-14

Figure 1 illustrates the effect of beta-glucans on the growth of *L. acidophilus* La-14. Eight different concentrations of beta-glucans were employed in this study which were 0, 2, 3, 4, 5, 6, 7 and 8% (w/v). Based on Figure 1, an increasing trend in the viability of *L. acidophilus* La-14 was observed as the concentration of beta-glucans increased. *Lactobacillus acidophilus* without the presence of beta-glucans (0% w/v) showed the lowest viability of *L. acidophilus* La-14 at 14.10 $\log_{10}$ CFU/mL. This result is in agreement with Savedboworn *et al.* (2017) who reported that probiotic *Lactobacillus plantarum* TISTR 2075 without prebiotic (inulin) showed the lowest viability as compared to probiotics with prebiotic.

On the other hand, the viability of *L. acidophilus* La-14 increases from 15.19 to 17.28 $\log_{10}$ CFU/mL as beta-glucans concentrations increases from 2 to 6% (w/v). However, 6 to 8% (w/v) of beta-glucans did not further increase ($p>0.05$) the viable cell counts of *L. acidophilus* La-14. This could be due to the high concentration of beta-glucans (7–8% w/v) was not able fully utilize by the probiotic within the 24 hours incubation. Hence, the highest viability of *L. acidophilus* La-14 was reported at all 3 beta-glucans concentrations (6, 7 and 8% w/v) with the range of 17.18–17.28 $\log_{10}$ CFU/mL. Similar results were reported by Siang *et al.* (2019) where the viable cell counts of *L. rhamnosus* GG increases as prebiotic isomalto-oligosaccharides concentration increases from 0 to 3.0% (w/v). Moreover, further addition of isomalto-oligosaccharides concentration (3 to 4% w/v) in the study also did not increase the viability of the probiotic.

In addition, the findings of this study were also in agreement with Gupta *et al.* (2017) where probiotics could acquire energy from prebiotics by consuming their end metabolic products such as acetate, propionate and butyrate. Both microbial-based beta-glucans with β-1,3, 1-6-glucans linkage and cereal-based β-1,3, 1-4-glucans can exert a positive effect on the modulation of the immune system (Rieder and Samuelsen, 2012). Besides that, beta-glucans can also form cylindrical molecules that consists approximately 0.25 million glucose units which could be utilized by probiotics as carbon source for growth (Lam and Cheung, 2013). In this study, due to similar viability ($p>0.05$) was displayed from 6 to 8% (w/v) of beta-glucans, 6% (w/v) of beta-glucans was chosen in this optimization for further analysis due to economic reasons.

Sequential digestion for *L. acidophilus* La-14 with or without beta-glucans

The viable cell counts of *L. acidophilus* La-14 with or without beta-glucans after incubated in SGJ and SIJ for 5 h were shown in Table 2. The viability of *L. acidophilus* La-14 with or without beta-glucans had a negative correlation with the simulated sequential digestion

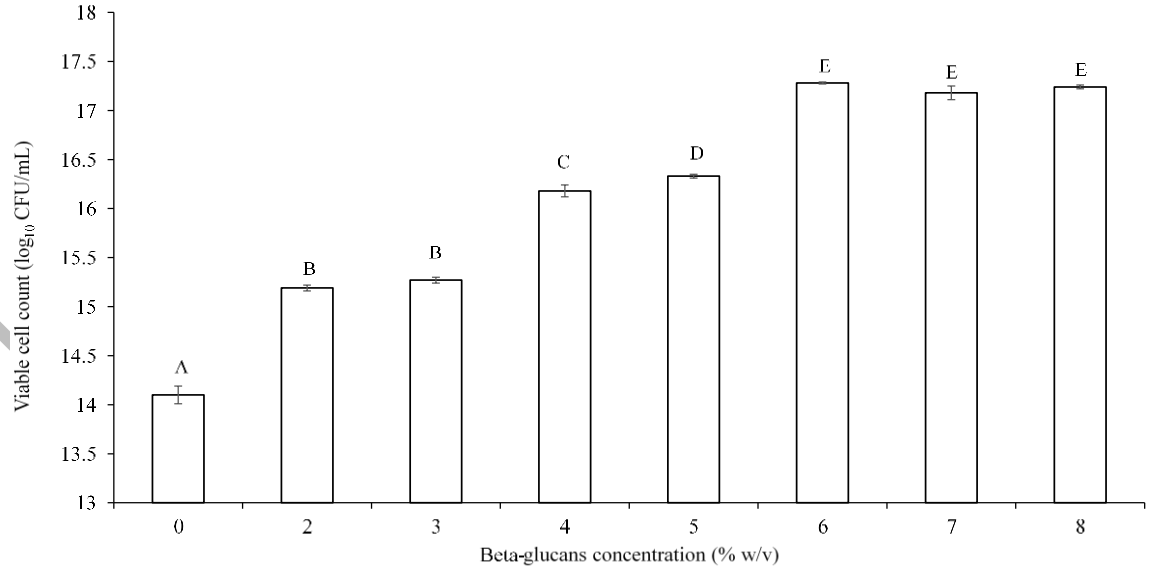

Figure 1: Effect of different beta-glucans concentrations on viable cell counts of *L. acidophilus* La-14. Error bars indicate the standard deviation of triplicate experiments (n=3). ^{ABCDE} means significantly different according to ANOVA and Tukey's post hoc test ($p \leq 0.05$).

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Table 2: Viable cell counts and survivability of *Lactobacillus acidophilus* La-14 with or without beta-glucans under simulated gastric juice (SGJ) and simulated intestinal juice (SIJ) incubation.

Sequential digestion	Time (h)	Viable cell counts (log_{10} CFU/mL)		Survivability (%)	
		L. acidophilus La-14	*L. acidophilus* La-14 with beta-glucans	*L. acidophilus* La-14	*L. acidophilus* La-14 with beta-glucans
SGJ (pH 2)	0	14.42 ± 0.09^{a}	15.68 ± 0.06^{a}	100.00 ± 0.00^{A}	100.00 ± 0.00^{A}
	1	13.69 ± 0.03^{b}	15.65 ± 0.04^{a}	94.94 ± 0.19^{B}	99.64 ± 0.38^{A}
	2	13.67 ± 0.07^{b}	12.62 ± 0.08^{b}	94.80 ± 0.48^{A}	80.48 ± 0.43^{B}
SIJ (pH 7.5)	3	11.63 ± 0.02^{c}	12.66 ± 0.03^{b}	80.65 ± 0.11^{A}	80.74 ± 0.38^{A}
	4	11.61 ± 0.04^{c}	12.65 ± 0.03^{b}	80.51 ± 0.25^{A}	80.68 ± 0.32^{A}
	5	11.67 ± 0.04^{c}	12.60 ± 0.06^{b}	80.93 ± 0.32^{A}	80.35 ± 0.39^{A}

^{abc} Means ± standard deviation followed by different superscript letters within the same column are significantly different according to ANOVA and Tukey's post hoc test ($p \leq 0.05$). ^{AB} Means ± standard deviation followed by different superscript letters within the same row are significantly different according to ANOVA and Tukey's post hoc test ($p \leq 0.05$).

incubation time. The viable cell counts of *L. acidophilus* La-14 without beta-glucans dropped by 5.06% after the first hour of incubation in SGJ. On the other hand, no reduction in viability was found ($p > 0.05$) in *L. acidophilus* with 6% (w/v) beta-glucans after 1 h of SGJ incubation. However, during the 2nd hour of SGJ incubation, the viability of *L. acidophilus* with beta-glucans decreased by 19.4%, while *L. acidophilus* without beta-glucans remained similar viable cell counts ($p > 0.05$). This showed that beta-glucans were not effective in protecting *L. acidophilus* during the simulated gastric environment. Similar findings were reported by Yee *et al.* (2019) where prebiotic (mannitol) did not exert a prebiotic effect on probiotic (*L. acidophilus* NCFM) throughout the 2 hours of SGJ incubation.

The viability of *L. acidophilus* La-14 without beta-glucans decreased by 14.9% during the first hour of SIJ incubation, while *L. acidophilus* La-14 with beta-glucans remained similar viability ($p > 0.05$). Nonetheless, the viability of both *L. acidophilus* La-14 with or without beta-glucans did not further reduce during the 2nd and 3rd hours of SIJ incubation. The reduction in viability during the first hour of SIJ incubation showed that *L. acidophilus* La-14 was sensitive to the alkaline environment and the presence of bile salt. However, *L. acidophilus* La-14 could be adapted to the stress alkaline environment with bile salt by releasing bile salt hydrolase after exposed to SIJ for 1 hour (Yeung *et al.*, 2016). According to Tanaka *et al.* (2012), exposing probiotics to stresses such as acidic, alkaline, temperature or bile salt at a milder or shorter period could enhance their survivability and tolerance towards these stresses.

Based on Table 2, *L. acidophilus* La-14 incorporated with or without 6% (w/v) beta-glucans demonstrated similar survivability of more than 80% at the end of the 5 h sequential digestion. This result demonstrated that *L. acidophilus* La-14 is a quality probiotic strain that could tolerate the environmental stress during the simulated digestive process, withstanding both stomach and intestinal pH with the presence of bile salt and digestive enzyme (Sahadeva *et al.*, 2011; Butel, 2014). Furthermore, a standard level of viable cell counts of 10^8–10^9 per mL is needed to have a positive effect on modulating gastrointestinal health (Albertini *et al.*, 2010).

Both *L. acidophilus* La-14 with or without beta-glucans fulfill the required viable cell counts after sequential digestion with 11.67 and 12.60 log_{10} CFU/mL, respectively (Table 2). This showed the acid and bile tolerance of the *L. acidophilus* La-14 strain. Although similar survivability was reported for both *L. acidophilus* La-14 with or without beta-glucans at the end of the 5 h sequential digestion, beta-glucans still showed to be an effective prebiotic in protecting *L. acidophilus* La-14 during the first hour of the simulated intestinal environment.

Viability of *L. acidophilus* La-14 with or without beta-glucans in tamarind juice during cold storage

Fruit juice is a potential vehicle for delivering probiotics due to its high nutritional value with no starter culture to compete with probiotics for growth (Nagpal *et al.*, 2012). Furthermore, oxygen scavenging components are often added into fruit juice during the manufacturing process. Therefore, processed fruit juice could often facilitate an anaerobic environment for *L. acidophilus* to grow as *L. acidophilus* is an facultatively anaerobic with optimal growth under microaerophilic conditions (Ding and Shah, 2008; Goldstein *et al.*, 2015). Table 3 shows the viability of *L. acidophilus* La-14 in different thermal-treated tamarind juice throughout 21 days of storage at 4 °C. All 5 samples of *L. acidophilus*-tamarind juice with or without beta-glucans reduced in viability throughout 21 days of cold storage.

The decreased in probiotics' viability could be due to the low pH condition of the tamarind juice (pH 3.17–3.63). According to Sheehan *et al.* (2007), the viability of probiotic (*L. paracasei*) in fruit juices is affected by pH lower than 4.5. Similar results were demonstrated by Nematollahi *et al.* (2016) and Mantzourani *et al*. (2018) where the viability of probiotics in different fruit juices reduced over cold storage. The low pH from the external

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Table 3: Viability of *Lactobacillus acidophilus* La-14 tamarind juice over 21 days of storage at 4 °C.

Storage time (day)	Viable cell counts (log_{10} CFU/mL)				
	S1	S2	S3	S4	S5
0	14.49 ± 0.04^{aA}	14.21 ± 0.07^{aA}	14.12 ± 0.03^{aA}	14.18 ± 0.05^{aA}	14.14 ± 0.01^{aA}
7	11.60 ± 0.03^{bA}	10.86 ± 0.06^{bB}	11.05 ± 0.05^{bA}	7.67 ± 0.10^{bC}	10.02 ± 0.02^{bD}
14	10.09 ± 0.08^{cA}	9.60 ± 0.08^{cC}	10.62 ± 0.04^{cB}	6.65 ± 0.02^{cE}	7.81 ± 0.01^{cD}
21	8.69 ± 0.08^{dA}	7.65 ± 0.16^{dB}	7.68 ± 0.07^{dB}	4.69 ± 0.11^{dD}	5.68 ± 0.13^{dC}

^{abcd} Means ± standard deviation followed by different superscript letters within the same column are significantly different according to ANOVA and Tukey's post hoc test ($p \leq 0.05$). ^{ABCDE} Means ± standard deviation followed by different superscript letters within the same row are significantly different according to ANOVA and Tukey's post hoc test ($p \leq 0.05$).
S1: *L. acidophilus*-tamarind juice with beta-glucans (without thermal treatment), S2: *L. acidophilus*-tamarind juice (thermal treatment 76 °C, 30 s), S3: *L. acidophilus*-tamarind juice with beta-glucans (thermal treatment 76 °C, 30 s), S4: *L. acidophilus*-tamarind juice (thermal treatment 90 °C, 60 s) and S5: *L. acidophilus*-tamarind juice with beta-glucans (thermal treatment 90 °C, 60 s).

environment could affect the survivability of probiotics by increasing the energy usage (ATP) to maintain the intracellular pH in the cells. This could further lead to insufficient ATP for other cellular activities, hence causing cell death (Nualkaekul *et al.*, 2011). Besides the pH of the fruit juice, the phytochemicals that are naturally present in tamarind juice may also exert antimicrobial activity towards the *L. acidophilus* over the storage period (Gupta *et al.*, 2014).

After 21 days of storage, S1 (tamarind juice without thermal treatment and incorporated with *L. acidophilus* La-14 and 6% w/v beta-glucans) displayed the highest viability (8.69 log_{10} CFU/mL) and the lowest reduction of viability 40.03% as compared to the thermal-treated tamarind samples (S2, S3, S4 and S5). This could be due to the phenolic compounds and phytonutrients in the juice affected by the thermal treatments which lead to the possible reduction of nutrients availability for probiotic growth (Indrawati *et al.*, 2004; Odriozola-Serrano *et al.*, 2008; Chaikham *et al.*, 2014; Petruzzi *et al.*, 2017). Moreover, the natural antioxidant compounds of the fruit juice such as vitamin C and anthocyanins could be degraded by the thermal treatment (Chaikham *et al.*, 2014; Petruzzi *et al.*, 2017). Therefore, the increase in oxidative stress could also reduce the viability of probiotics (Odriozola-Serrano *et al.*, 2008).

The prebiotic effect of beta-glucans towards *L. acidophilus* La-14 over the 21 days of cold storage was shown in Table 3. There was no difference in viability found between S2 and S3 (76 °C for 30 s). On the other hand, *L. acidophilus* with beta-glucans in the thermal-treated tamarind juice (90 °C for 60 s) (S5) showed higher viability (5.68 log_{10} CFU/mL) and a lower reduction in viability (59.8%) than S4 (without beta-glucans) (4.69 log_{10} CFU/mL) after 21 days of cold storage. This shows that beta-glucans were able to act as a nutrient source for *L. acidophilus* La-14 in thermal-treated tamarind juice (90°C for 60 s). The high thermal treatment could have negative effect on the nutrients in the tamarind juice (Indrawati *et al.*, 2004; Odriozola-Serrano *et al.*, 2008). Hence, the addition of prebiotics can facilitate the viability of probiotics caused by acidity reduction, changes in temperature, deficient nutrients and oxidative activity (Lam and Cheung, 2013).

The final viable cell counts of *L. acidophilus* La-14 at the end of the 21 days storage for S1, S2 and S3 was more than 7 log_{10} CFU/mL which fulfills the minimum requirement of probiotic cells in food after storage (Hill *et al.*, 2014). However, S4 and S5 did not meet the viability requirement with only 4.69 and 5.68 log_{10} CFU/mL after 21 days of storage. This indicated that thermal treatment at 90 °C for 60 s was not suitable for the processing of tamarind juice incorporated with *L. acidophilus*.

Physicochemical properties of *L. acidophilus* La-14 tamarind juice with or without beta-glucans during cold storage

Figure 2 shows the physiochemical changes (pH, titratable acidity, total solid content and viscosity) of *L. acidophilus* La-14 tamarind juice with or without beta-glucans during cold storage. The pH and titratable acidity of the *L. acidophilus* La-14 tamarind juice (S1, S2, S3, S4 and S5) remained constant ($p>0.05$) throughout the 21 days of storage (Figure 2a and 2b). Fermentation is a phenomenon where probiotics could utilize sugar in fruit juice as a nutrient for growth. The organic acids produced during fermentation would decrease the pH and increase the titratable acidity of the product (Ding and Shah, 2008). However, no changes in pH or titratable acidity were observed in this study which indicates that no probiotic fermentation had occurred in the tamarind juice over 21 days of storage at 4 °C. Similar results were reported by Nematollahi *et al.* (2016) where the pH and titratable acidity of probiotic (*L. rhamnosus* or *L. plantarum*)-cornelian juice remained unchanged throughout 21 days of storage at 4 °C.

According to Ferdousi *et al.* (2013) and Savedboworn *et al.* (2019), probiotics stored under cold temperature have low cell metabolism, hence preventing the occurrence of cell activity such as the utilization of nutrients. As changes in pH and titratable acidity would affect the taste of the probiotic-fruit juice (sourness) (Mostafa *et al.*, 2021), maintaining the pH and titratable acidity throughout the storage period is crucial to provide a consistent taste of the product towards consumers. The cold temperature could offer *L. acidophilus*-tamarind juice more stable sensory properties in terms of taste.

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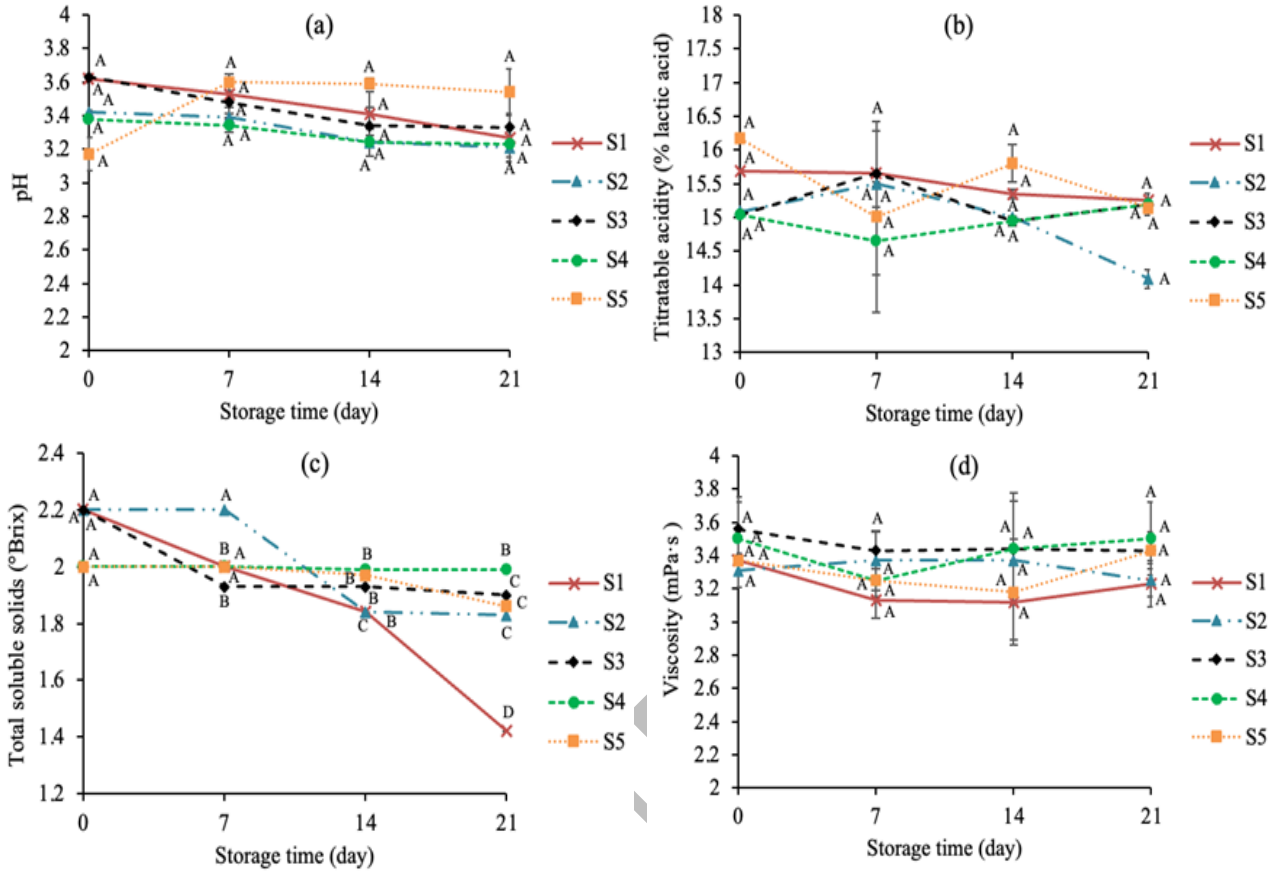

Figure 2: Physicochemical properties of *L. acidophilus*-tamarind juice over 21 days of cold storage. (a) pH, (b) titratable acidity, (c) total soluble solids and (d) viscosity. S1: *L. acidophilus*-tamarind juice with beta-glucans (without thermal treatment), S2: *L. acidophilus*-tamarind juice (thermal treatment 76 °C, 30 s), S3: *L. acidophilus*-tamarind juice with beta-glucans (thermal treatment 76 °C, 30 s), S4: *L. acidophilus*-tamarind juice (thermal treatment 90 °C, 60 s) and S5: *L. acidophilus*-tamarind juice with beta-glucans (thermal treatment 90°C, 60 s). Error bars indicate the standard deviation of triplicate experiments (n=3). ^{ABCD} means significantly different over 21 days of cold storage (at 4 °C) according to ANOVA and Tukey's post hoc test ($p \leq 0.05$).

On the other hand, total soluble solids of *L. acidophilus*-tamarind juice were reduced over 21 days of cold storage (Figure 2c). Similar results were reported by Mokhtari *et al.* (2019) and Afzaal *et al.* (2020) where total soluble solids of probiotic-fruit juice reduced over storage at 4 °C. Both studies explained that the reduction of total soluble solids was due to the utilization of sugar by probiotic during storage. However, as no reduction in pH and titratable acidity was found during the 21 days of storage in this study, therefore the reduction of total soluble solids was not due to the utilization of sugars by probiotics for growth. Non-enzymatic browning reaction is a natural chemical reaction that could occur in fruit juices upon prolonged storage. This natural phenomenon could contribute to the reduction of total soluble solids as sugar was degraded (Bharate and Bharate, 2014). Similar observation was reported by Lu *et al.* (2019) where the soluble sugar decreased upon storage.

Viscosity is one of the important parameters in evaluating beverage products as it is highly related to the structural composition of the beverage (Damin *et al.*, 2008). The viscosity of *L. acidophilus*-tamarind juice in this study remained unchanged throughout 21 days of cold storage ($p>0.05$) (Figure 2d). According to Garcia *et al.* (2020) and Deepak *et al.* (2016), certain lactic acid bacteria such as *L. acidophilus* could produce exopolysaccharides (EPS) that would increase the viscosity of fruit juice during fermentation. However, as pH and titratable acidity of the *L. acidophilus*-tamarind juice remained unchanged in this study, it is assumed that no fermentation had occurred. Hence, the viscosity of the *L. acidophilus*-tamarind juice remained the same throughout the cold storage. This shows that storing *L. acidophilus*-tamarind juice at 4 °C could maintain the stability of the beverage in terms of viscosity.

Based on Figure 3, the color changes in *L. acidophilus*-tamarind juice increased over 21 days of storage at 4 °C. According to Mubaiwa *et al.* (2018), the interpretation of total color changes less than 1 indicates that the color changes are not perceptible by the human

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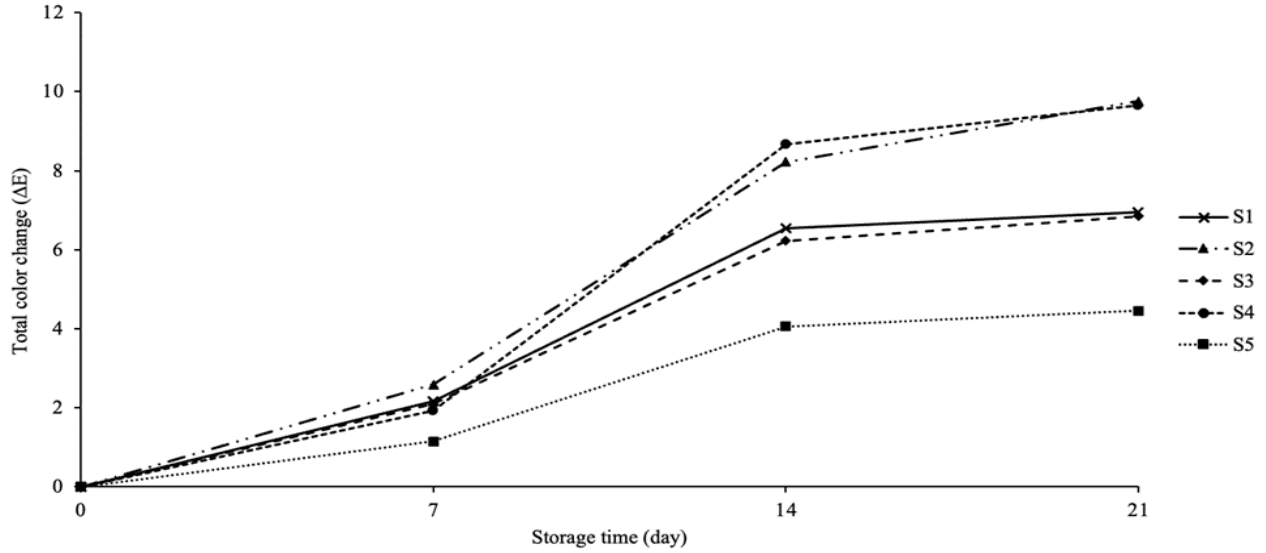

Figure 3: Total color change (ΔE) of *L. acidophilus*-tamarind juice over 21 days of cold storage at 4 °C. S1: *L. acidophilus*-tamarind juice with beta-glucans (without thermal treatment), S2: *L. acidophilus*-tamarind juice (thermal treatment 76 °C, 30 s), S3: *L. acidophilus*-tamarind juice with beta-glucans (thermal treatment 76 °C, 30 s), S4: *L. acidophilus*-tamarind juice (thermal treatment 90 °C, 60 s) and S5: *L. acidophilus*-tamarind juice with beta-glucans (thermal treatment 90 °C, 60 s).

eye, while value ranges between 1–2 means that the color changes are perceptible by the human eye via a close observation. On the other hand, total color changes locate between 2 to 10 shows that color changes can be sensed through a glance. After 7 days of storage, color change perceptible by the human eye through close observation was detected in S4 and S5 (1.92 and 1.15, respectively) which are tamarind juice that was heated at 90 °C for 60 s, while S1, S2 and S3 had higher color change (2.17, 2.59 and 2.09, respectively) that can be sensed through a glance. The higher stability in color change displayed by tamarind juice with higher heating temperature and duration (90 °C for 60 s) could be due to partial inactivation of enzyme present in tamarind juice that could lead to browning.

All 5 *L. acidophilus*-tamarind juice samples demonstrated color changes that can be sensed through a glance after 21 days of storage. This could be due to the breakdown of particles in tamarind juice by enzymes released by both viable and non-viable *L. acidophilus* throughout 21 days of storage (Ding and Shah, 2008; Adekunte *et al*., 2010). Nevertheless, the least color change was displayed by S5 thermal-treated tamarind juice (90 °C for 60 s) with the presence of *L. acidophilus* La-14 and beta-glucans. This shows that both thermal treatment and prebiotic beta-glucans could contribute to the stability of color during storage. Similar results were also reported by Renuka *et al.* (2009) where fortifying fruit juices with prebiotic fructo-oligosaccharide (FOS) were able to retain the color of the fruit juices throughout storage.

CONCLUSION

In conclusion, 6% (w/v) of beta-glucans with *L. acidophilus* La-14 displayed the highest viability (17.28 $\log_{10}$ CFU/mL). Both *L. acidophilus* La-14 with or without beta-glucans was able to survive more than 80% after 5 h of sequential digestion. Furthermore, beta-glucans were able to protect the viability of *L. acidophilus* La-14 in tamarind juice with thermal treatment (90 °C for 60 s) throughout 21 days of cold storage. However, thermal treatment of 76 °C for 30 s was more suitable for tamarind juice incorporated with *L. acidophilus* La-14 than 90 °C for 60 s as they met the minimum requirement of 6 $\log_{10}$ CFU/mL after 21 days of storage. Cold storage (4 °C) of *L. acidophilus* La-14 tamarind juices effectively retained the pH, titratable acidity and viscosity after 21 days of storage. On the other hand, thermal treatment contributed to the stability of total soluble solids in *L. acidophilus* La-14 tamarind juices; while the combination of beta-glucans and thermal treatment was able to maintain the color stability of the *L. acidophilus* La-14 tamarind juice. Thermal-treated tamarind juice is a potential non-dairy beverage in delivering probiotic *L. acidophilus* La-14. Future studies could further explore the functionality and the sensory properties of thermal-treated tamarind juice with *L. acidophilus* La-14 and beta-glucans.

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