Microbial, Physiochemical and Sensory Properties of Bael (*Aegle marmelos*) incorporated Buffalo Milk Yoghurt containing *Lb. rhamnosus* GG

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Abstract: Recently, there is a trend among food manufacturers in producing functional foods containing fruit and milk. On the other hand, there is an increasing demand for buffalo milk as an alternative to cow milk. Therefore, the aim of this study was to investigate the impact of adding bael fruit extract on microbial, physiochemical and sensory characteristics of buffalo yoghurt containing the EPS-producing probiotic *Lactobacillus rhamnosus* GG (LGG) during 21 days of refrigerated storage. Four formulations of buffalo yoghurt: a control yoghurt manufactured with conventional yoghurt culture (CON); a probiotic yoghurt containing *Lb. rhamnosus* GG plus yoghurt culture (PY); a probiotic yoghurt containing 5% (w/v) bael (PY5); and 10% (w/v) bael (PY10) were evaluated for changes in pH, syneresis, hardness, probiotic viability and sensory attributes during the storage. Control yoghurt experienced significant post acidification and a higher rate of syneresis. Addition of probiotics had a positive effect on post acidification and syneresis rate. Addition of bael did not affect post-acidification, but significantly decreased the level of syneresis. All probiotic formulations maintained LGG counts of >10^7 cfu/mL and the highest counts were observed in 5% (w/v) bael incorporated yoghurt. Results showed that buffalo yoghurt is an ideal matrix to deliver LGG and 5% bael incorporation would be ideal or symbiotic product development.

Keywords: bael; buffalo milk; *Lactobacillus rhamnosus* GG; non-cow milk; probiotic yoghurt; probiotic viability

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1. Introduction

Probiotics are live microorganisms, which when administered in adequate amounts confer health benefits on the host [1]. It is widely accepted that at least 10^6-10^9 cfu/mL or g of viable probiotic cells must be available in the final product at the time of consumption to assure any therapeutic effect [2]. Upon ingestion, these cells must survive the passage through the human gastrointestinal (GI) tract, enter the colon at sufficient quantities (10^9 cfu/day), adhere to, and colonize the gut epithelium [3,4]. Regular consumption of probiotics increases the relative numbers of beneficial bacteria in the colon, which in turn impose a positive impact on immune function, digestion, metabolism, and brain-gut communication [5]. Food manufacturers are attracted to probiotics due to the projected market growth, high margins and growing consumer demand for functional foods [6].

Fermented dairy products including fermented milk, yoghurt and cheese have traditionally been used as vehicles for probiotic delivery [4,6]. Due to the buffering capacity of milk, the dairy matrix offers greater survivability to probiotic microorganisms during processing, storage and gastrointestinal transition [6,7]. Therefore, dairy products remain at the forefront of commercial probiotic food development and are becoming popular due to their health-promoting effects [8,9].

Yoghurt is one of the most popular dairy products in the world due to its flavor, nutritional characteristics and well-known health benefits [10]. Yoghurt and yoghurt-like products are considered the best known-food vehicle for probiotics [2]. In addition, both the starter culture and probiotic microorganisms can be included into yoghurt giving rise to many different microbial combinations [7]. Cow milk is widely utilized for yoghurt manufacture all over the world whereas, milk from other species including goat and sheep are also utilized to a lesser extent.

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There is increasing popularity for buffalo milk products as a specialty and an alternative to cow milk [11]. Formulation of fermented buffalo milk products may expand the range of products with different qualities than those traditionally made with cow milk [11,12]. Buffalo milk contains higher levels of total solids (16-19%) than cow milk, which can give better textural characteristics to yoghurt [10]. Moreover, it contains higher levels of fat (6.5–8.0%), protein (4.59–5.37%), lactose (4.49-4.73%), and certain minerals (Ca, Fe, Mg, P) and contain almost double the content of Conjugated Linoleic Acid (CLA) [12,13]. Greater quantities of caseins and fat of buffalo milk provide the final product with a better gel consistency and more creaminess, respectively [12]. Mounting evidence suggest that buffalo milk is an adequate matrix to deliver probiotics and to develop synbiotic products [11,12,14,15].

Lactic Acid Bacteria (LAB) mainly from genera *Lactobacillus*, *Bifidobacterium*, *Streptococcus* and *Enterococcus* have widely been utilized for probiotic food development currently [3,16]. There is an increasing trend in the use of exopolysaccharide (EPS)-producing starter cultures due to their water-binding and texture promoting abilities [17]. *Lactobacillus rhamnosus* GG is one of those EPS-producing probiotic bacteria which is referred to as the most studied *Lactobacillus* strain for human applications [18–20]. It has been successfully employed in the manufacture of probiotic dairy products including fermented milk and yoghurts [19,21,22]. Health benefits reported for *Lb. rhamnosus* fermented milk products include prevention and reduction of the duration of diarrhea [23,24], alleviation of skin conditions such as eczema and allergic reactions [25,26], enhanced immunity against dental caries [27], and reduced risk of urogenital infections [19]. Moreover, many research has shown that *Lb. rhamnosus* is well-survived in fermented milk products containing fruits and fruit juices [28–33].

Recently, there is a trend among food manufacturers in producing functional foods containing fruit and milk [6]. However, publications on dairy products enriched with fruity bases and possible impacts of these fruits on the viability of the probiotics in the food product are scarce [2]. Bael (*Aegle marmelos*) is a subtropical tree found in the Indian subcontinent. Its leaves, fruits, flowers, seeds, roots and bark are long been used in traditional medicine to treat myriad ailments, chronic diarrhea, dysentery, and peptic ulcers [34,35]. Enormous medicinal benefits of bael have recently been reviewed [34]. Bael fruit pulp contains approximately 0.27% fat, 1.12% protein and 3.3% fiber in addition to many other functional and bioactive compounds including carotenoids, phenolics, alkaloids, and flavonoids [34,36]. The fruit pulp of bael is used to prepare delicacies such as murabba, puddings and juice [35]. Therefore, incorporating bael into buffalo milk matrix would be an ideal blend that combines the goodness of both into a single product.

Based on this background, the aim of the current study was to determine the effects of adding bael fruit extract on some microbiological, physiochemical and sensory properties of buffalo milk yoghurt containing EPS-producing probiotic strain *Lb. rhamnosus* GG.

2. Materials and Methods

2.1 Preparation of Bael Fruit Extract

Fully ripen bael fruits were used to prepare the water extract of bael. The pulp was extracted by breaking the outer shell and mixed with sterilized water in 1:1 ratio. Then it was thoroughly stirred until the clumps were disappeared. The extract was pasteurized (at 60 °C for 5 minutes), cooled down to room temperature, stored in a glass container and kept under refrigerated conditions (4±1 °C) until use.

2.2 Preparation of Starter Cultures

Freeze-dried yoghurt starter culture (YF-L811) and probiotic *Lb. rhamnosus* GG (nu-trish® LGG®) were obtained from Christian Hansen, Horsholm, Denmark. The yoghurt starter culture contained a mixture of *Streptococcus thermophilus* and *Lactobacillus delbrueckii* ssp. *bulgaricus* in 1:1

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ratio. Yoghurt starter and probiotic cultures were activated by inoculating 0.05% (w/v) of the lyophilized granules with 100 mL portions of pasteurized skim cow milk separately. The yoghurt starter culture and probiotic culture were incubated overnight at 42 °C and 37 °C, respectively. Then the working cultures were prepared by inoculating skim cow milk with 5% (v/v) of the activated cultures which were incubated at the above mentioned temperatures for 18 h. These cultures were used to inoculate yoghurt milks that were used to prepare experimental yoghurts in the current study.

2.3 Preparation of Yoghurts

We tested four different formulations of buffalo milk yoghurt: a control yoghurt manufactured with conventional yoghurt culture (CON); a probiotic yoghurt containing *Lb. rhamnosus* GG (PY); a probiotic yoghurt containing *Lb. rhamnosus* GG and 5% (w/v) bael extract (PY5); and a probiotic yoghurt containing *Lb. rhamnosus* GG and 10% (w/v) bael extract (PY10).

Fresh and full-fat buffalo milk was purchased from Mahayaya Farm located in the Northwestern Province of Sri Lanka during September and October 2020. To prepare a batch of yoghurt formulations, a portion of buffalo milk was skimmed at 40 °C using a laboratory-scale centrifugal cream separator. Then the full-fat buffalo milk was standardized to a 5% fat level by mixing with skimmed buffalo milk. The standardized buffalo milk was preheated to 60 °C and homogenized (Dragon Laboratory Instruments Limited, Beijing, China). The homogenized mixture was pasteurized at 80-85 °C for 25 min with constant stirring while adding 0.25% (w/v) gelatin and 4% (w/v) sucrose. The total volume of milk was divided into four equal portions assigned to the four treatments (CON, PY, PY5 and PY10) and cooled down to 42 °C. Each portion was inoculated with previously activated cultures according to the ratios mentioned in the Table 1. To manufacture probiotic yoghurts (PY, PY5 and PY10), *Lb. rhamnosus* GG was inoculated at a concentration of 2% (v/v) of the total milk volume. Bael-incorporated probiotic yoghurts were manufactured by adding 5% (w/v) and 10% (w/v) of bael fruit extract into yoghurt milks assigned for PY5 and PY10. Yoghurt milks were thoroughly mixed, poured into 85g standard polystyrene yoghurt cups and incubated at at 42 °C until a pH of 4.5-4.8 reached. After that all yogurt samples were stored under refrigerated conditions (4±1 °C). During the study period, several batches of yoghurts were routinely prepared following the same methodology.

Table 1. Inoculating ratios of conventional yoghurt starter, probiotic culture and bael fruit extract.

Treatment	Yoghurt starter culture % (v/v)	Probiotic culture % (v/v)	Bael extract % (w/v)
CON	4	0	0
PY	2	2	0
PY5	2	2	5
PY10	2	2	10

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2.4 Determination of Physiochemical Properties

The physiochemical parameters of experimental yoghurt preparations were determined at 1, 7, 14 and 21 d of refrigerated storage in triplicates.

Measurement of pH

The pH values were determined according to the AOAC protocols [37] using a digital pH meter (OHAUS, STARTER 3000, US). The pH was measured just after the preparation of yoghurts, after 2.5 h of fermentation and then at 30 min intervals until the pH reaches 4.5-4.8. In addition, it was determined at aforementioned time points during cold storage.

Measurement of Syneresis

Syneresis of the yoghurt samples during refrigerated storage was measured according to the method previously described by Gursel et al. [10] with slight modifications. Briefly, 15 g of yoghurt samples were centrifuged at 640 × *g* for 20 min at 4 °C using a bench top centrifuge (Digicen 21 R, Orto Alresa, Spain). Syneresis (%) was calculated using the following equation.

$$Syneresis\ (\%) = \frac{Volume\ of\ whey\ separated\ (mL)}{Sample\ weight\ (g)} \times 100$$

Texture analysis

The texture analysis was performed using a texture analyzer (Shimadzu, Kyoto, Japan) equipped with 50 kg load cell and a piercing test jig (3 mm in diameter). Hardness was the texture parameter analyzed by a piercing and penetration test. The test was performed directly in standard plastic containers each filled with 70 mL of yoghurt. The probe was moved at a test speed of $1 mm.s^{-1}$ from the yoghurt surface until a depth of 18 mm into the yoghurt was reached. Three (03) measurements at different positions were made with each of the samples. Force and the time data was recorded using a texture analyzing software (Trapezium X, Shimadzu, Japan). Testing was conducted immediately after removing the samples from the refrigerator.

2.5 Determination of Probiotic Viability

One gram (1 g) of yoghurt was suspended in 9 mL of 1% (w/v) peptone water and subsequently 10-fold serially diluted to 10^{-6}. Selective enumeration of *Lb. rhamnosus GG* was carried out using the pour plate technique after anaerobic incubation at 37 °C for 72 h on MRS vancomycin media (pH 6.2). The media contained 50 ppm vancomycin (GUFIC®, Gujarat, India) and the anaerobic conditions were created using anaerobic sachets (Microbiology Anaerocult® C, Darmstadt, Germany). *Lb. rhamnosus* GG colonies were confirmed by white, shiny and smooth colonies approximately 2 mm in diameter [38]. Plates containing 20 to 200 colonies were enumerated using a colony counter (Stuart SC6PLUS, Stuart Scientific, UK) and recorded as colony forming-units per gram (cfu/g) of the product.

2.6 Determination of Sensory Characteristics

Sensory evaluation of the four yoghurt formulations was conducted by 34 untrained taste panelists aged 23-40. Panelists were recruited from the Faculty of Livestock, Fisheries and Nutrition,

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Wayamba University of Sri Lanka. All the panelists were regular consumers of yoghurt and selected based on interest, time availability, non-smoking status and lack of food allergies. Panelists evaluated four yoghurt samples in individual boots under controlled lighting and temperature. During the evaluation, no verbal communication was allowed among the panelist to ensure accurate data collection. Forty grams (40 g) of each sample were served in uniform plastic containers labeled with a random 3-digit code. The samples were presented in a randomized order among the panelists and were instructed to rinse before tasting each sample. Each attribute (appearance, color, aroma, texture, taste, after-taste, mouthfeel and overall acceptability) was evaluated on a 7-point hedonic scale (7 = like very much; 4 = neither like nor dislike; 1 = dislike very much).

2.7 Statistical Analysis

Results were reported as mean ± standard deviation (SD) for the triplicate analyses. A two-way mixed ANOVA was used to determine main effects of treatment (between-subject factor) and storage time (within-subject factor) on physiochemical parameters (pH, syneresis and hardness) and probiotic viability. Means were separated using Tukey Test. Data were submitted to statistical analysis as randomized complete blocks arranged in a 4 × 4 factorial scheme (4 treatments: C, PY, PY5, and PY10; and four lengths of storage time; 1, 7, 14 and 21 d). Multiple Repeated Measures ANOVA and separate One-way ANOVAs were performed to determine the 'simple main effect for time' and 'simple main effect for treatment' on physiochemical and microbial viability data, respectively. Whereas, sensory data were analyzed using the Friedman test and separate Wilcoxon signed-rank tests were performed to analyze differences among the groups. All statistical analyses were performed using SPSS version 22.0 (SPSS Inc., Chicago, IL) with the significance level at $p<0.05$).

3. Results and Discussion

3.1. Post-acidification

Buffalo milk and bael used to prepare yoghurt formulations had a pH of 6.2-6.3 and 5.0-5.5, respectively. The initial pH of the yoghurt formulations was ranged from 6.08-6.19 and the highest pH was observed in standard yoghurt (6.19±0.03). As expected, the addition of bael extract resulted in a slight reduction of the initial pH in yoghurt formulations containing bael. Inherent acidity of the bael extract comes from naturally occurring organic acids such as oxalic, malic and tartaric acids [34]. Similar results were reported by Ghadge et al. [39] where the pH of plain buffalo yoghurt (4.39±0.10) was decreased to 4.10 and 4.27 after incorporating honey and apple pulp, respectively [39]. During fermentation, the initial pH of the buffalo yoghurt formulations was continuously decreased and gave rise to products having a final pH of 4.5-4.8. Then the products were stored under refrigerated conditions (4±1 °C) where the post acidification was monitored in terms of pH at 1, 7, 14 and 21 days of refrigerated storage which has been depicted in the Figure 1.

Statistical analysis revealed that there was an interaction effect between treatment and storage time on pH ($p<0.005$). Changes in acidity and pH during storage are associated with the growth and metabolism of LAB present in the product that breakdown carbohydrates into organic acids. Increase in acidity due to the continuous production of organic acids is referred to as post-acidification, which cause a drop in pH during storage [40,41]. In the current study, control yoghurt experienced a significant pH drop (0.223 units) over the 21 d of refrigerated storage. In contrast, there was no significant change in the pH values of the yoghurts containing probiotics (PY, PY5 and PY10). Stable

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pH observed in the probiotic yoghurts for over the refrigerated storage may be due to the buffering ability of the EPS produced by *Lb. rhamnosus* GG. Similar findings were reported for milk-juice beverages with fermented sheep milk and strawberry where the post acidification found to be significantly low in products manufactured with probiotic *Lb. plantarum* compared to those manufactured with conventional yoghurt bacteria [42]. Furthermore, Akgun et al. [15] reported that post-acidification of buffalo milk yoghurt fermented by traditional yoghurt cultures containing *St. thermophilus* and *Lb. delbruekii* subsp. *bulgaricus* was significantly higher than the probiotic ones. The post-acidification observed in the current research was considerably lower than the data reported for buffalo milk yoghurt in the available literature. For example, a post-acidification rate of 4.53-3.93 was observed in a symbiotic buffalo yoghurt containing *Lb. acidophilus* and *B. bifidum* over 21 d of refrigerated storage [11].

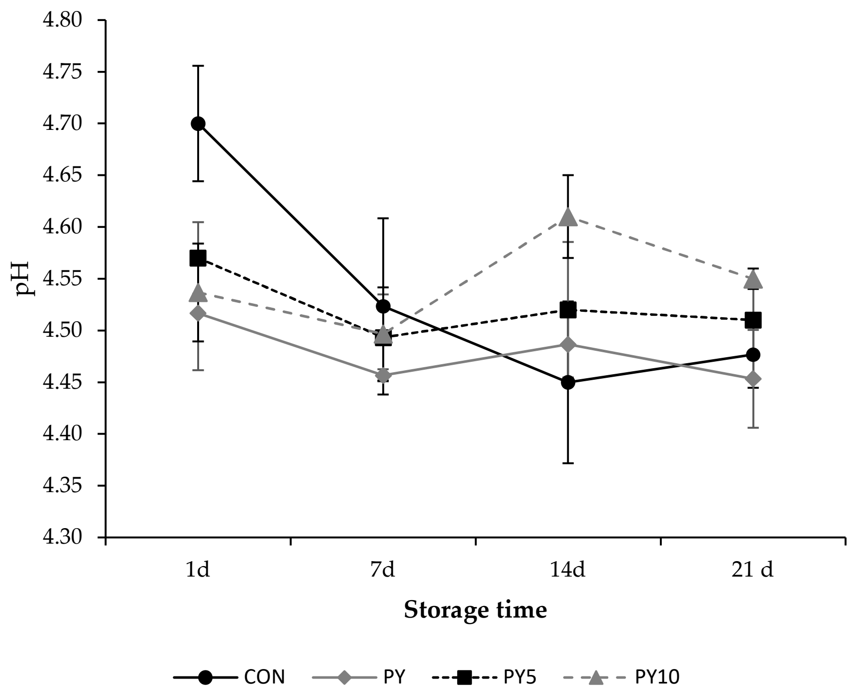

Figure 1. pH changes during refrigerated storage (4 °C) of buffalo milk yoghurt preparations: CON = control yoghurt produced with conventional starter cultures; PY = probiotic yoghurt containing *Lb. rhamnosus* GG; PY5 = probiotic yoghurt containing *Lb. rhamnosus* GG and 5% (w/v) bael extract; and PY10 = probiotic yoghurt containing *Lb. rhamnosus* GG and 5% (w/v) bael extract. Values are mean ± SD.

Another interesting observation in the post-acidification profiles was that there was a hike in pH of the probiotic yoghurts at 14th day of the storage while the pH in the control yoghurt was continuously decreasing. This hike was correlated with the viable probiotic counts at the 14th day as the magnitude of the pH increase was proportionated to the viable probiotic counts present in the respective treatment. For example, the greatest pH increase was observed in the PY10 which had the highest viable probiotic count (1.96×10^8 cfu/g), and the lowest hike was observed in the PY which had the lowest viable probiotic count (1.77×10^7). Malaka et al. (2019) observed that the pH of an acid milk curd fermented by *Lb. bulgaricus* was increasing with increasing levels of EPS [43]. Therefore, it is likely that the pH hike observed at 14th day of the storage in the current study was due to the higher production of EPS by *Lb. rhamnosus* GG. Similar results were reported for acai yoghurt in which a subsequent increase in pH was observed at 21 and 28 d of storage after observing a significant reduction in pH values during the first 14 d of storage [40].

Moreover, another drop in pH was observed in the probiotic yoghurts after 21 d of storage. This may be due to the accumulation of organic acids produced by *Lb. rhamnosus* GG as a result of the greater viable counts seen at the 14 d of cold storage. Another important observation of the current research is that the pH in bael incorporated yoghurts (PY5 and PY10) between 14-21 d of the refrigerated storage were greater than those without bael (CON and PY). Moreover, the acidity was tended to increase with increasing level of fortification. This is likely to be associated with the higher viable counts observed in bael incorporated yoghurts as beal may provide additional fermentable substrates that promote the growth of LAB. Similar trends in pH were observed in fenugreek seed flour-incorporated buffalo yoghurt [41], honey or apple pulp incorporated buffalo yoghurt [39], stingless bee honey-incorporated goat milk yoghurt [40] and mango juice-enriched probiotic dairy drinks [6].

3.2. Syneresis

Syneresis is the expulsion of whey from the network which then become visible as surface whey, and is a major quality defect in yoghurts that negatively affects consumer perception [44,45]. Previous research suggests that homogenization of buffalo milk result in significantly lower syneresis and improved gel firmness in buffalo milk yoghurt during cold storage compared to those manufactured using unhomogenized milk [44,46]. Therefore, we have used homogenized buffalo milk to manufacture experimental yoghurt preparations in the current study.

Syneresis in different yoghurt formulations over the refrigerated storage is depicted in Figure 2. Statistical analysis revealed that both the treatment and storage time significantly influence syneresis in the buffalo milk yoghurts ($p<0.001$). Between 1-21 d of storage, the control yoghurt experienced a 12% increase in syneresis ($p<0.05$). In contrast, PY, PY5 and PY10 showed a 4%, 36% and 30% decline in syneresis, respectively. Accordingly, after 21 d of storage, the highest syneresis value was observed for the control yoghurt (38.66±1.34%). A similar level of syneresis (39–42%) has previously been reported by Akgun et al. [15] for buffalo milk yoghurt during cold storage. Previous research findings suggest that an increase in acidity may increase syneresis in buffalo milk yoghurt during the cold storage [39]. Therefore, the significant increase in syneresis observed in the control yoghurt may be due to the significantly higher post-acidification occurred during storage.

After 21 d of refrigerated storage, the syneresis in plain probiotic yoghurt (PY) was significantly lower ($p<0.05$) than that of the control yoghurt suggesting that the addition of *Lb. rhamnosus* GG resulted in a significantly reduced syneresis in buffalo milk yoghurt. It has been reported that both the higher water-binding capacity and the modification of yoghurt microstructure by EPS affect syneresis [17]. Therefore, it is likely that the EPS produced by *Lb. rhamnosus* GG is responsible for the reduced level of syneresis observed in probiotic yoghurt in the current study. Similar results have previously been reported by Amatayakul et al. [17] where the yoghurts made with EPS-producing starter culture had a lower level of syneresis than yoghurt produced with non-EPS producing starter cultures.

Our results showed that incorporation of bael extract significantly reduced syneresis in bael-incorporated buffalo milk yoghurts and showed the lowest level of syneresis (<20%) after 21 d of cold storage. Previous literature on buffalo milk yoghurt suggests that the addition of prebiotics significantly reduced syneresis by increasing the water-binding ability [11]. Therefore, it is likely that bael extract exerts possible prebiotic effect which in turn resulted in significantly low levels of syneresis in bael-incorporated yoghurt. Moreover, the level of syneresis in the 5% bael – incorporated and 10% bael incorporated yoghurts were comparable (p>0.05).

One interesting observation during the storage was that there was a drastic increase in syneresis in all four treatments after 14 d of storage compared to 7d of storage. This may be due to the structural rearrangements in casein-micelles in the gel network and the rate of solubilization of calcium during the storage [15].

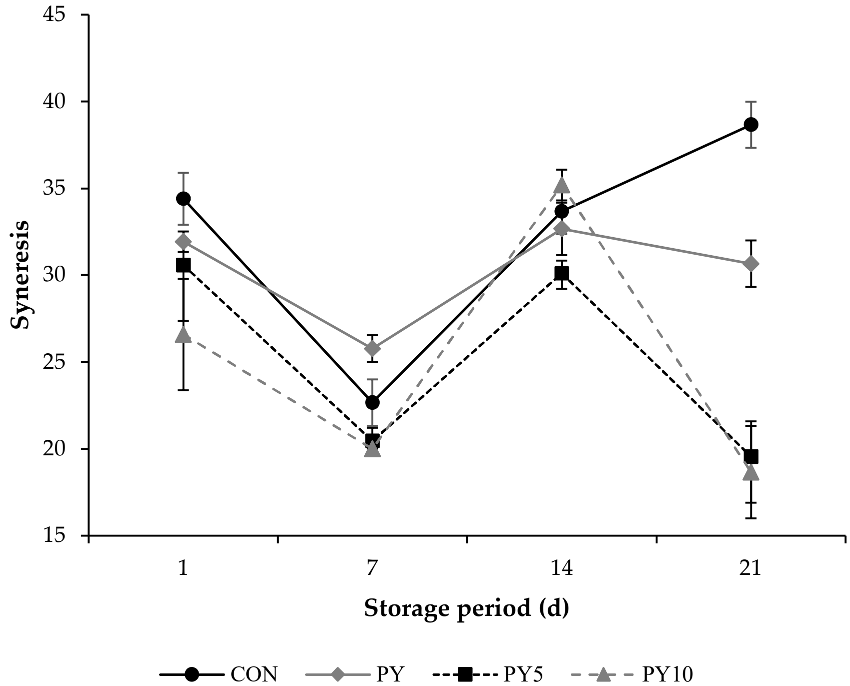

Figure 2. Syneresis changes during refrigerated storage (4 °C) of buffalo milk yoghurt preparations: CON = control yoghurt produced with conventional starter cultures; PY = probiotic yoghurt containing *Lb. rhamnosus* GG; PY5 = probiotic yoghurt containing *Lb. rhamnosus* GG and 5% (w/v) bael extract; and PY10 = probiotic yoghurt containing *Lb. rhamnosus* GG and 5% (w/v) bael extract. Values are mean ± SD.

3.3. Hardness

Texture is a key determinant of yoghurt quality and is affect appearance, mouthfeel and overall acceptability [47]. Out of different textural parameters, hardness is the most important parameter for evaluation of yoghurt texture and is also considered as a measure of yoghurt firmness [48]. The hardness values for different yoghurt formulations during 21 d at 4 °C are presented in Table 2. Results showed that both storage time and treatment have a significant influence on hardness values (p<0.05). In general, there was an increasing trend in hardness values among the treatments with increasing storage time. Similar results were previously reported by Cui et al. [49] for cow milk yoghurt stored for 28 d. One interesting observation throughout the storage was that the hardness values for probiotic yoghurts (PY, PY5 and PY10) were relatively higher than the control yoghurt. In a previous study, *Lb. rhamnosus* GG reported producing EPS which could improve the texture of yoghurt by interacting with the free-water in the gel structure [49]. In another study, the firmness of yoghurts made using capsular EPS- or ropy EPS-producing starter cultures was generally lower than that in yoghurts produced with non-EPS-producing starter cultures [17]. These observations suggest that EPS produced by EPS-producing probiotic strains could increase the hardness of yoghurt during storage. Therefore, it seems that the EPS produced by *Lb. rhamnosus* GG was responsible for the higher hardness observed among the probiotic yoghurts. Moreover, after 21 d of storage, the lowest hardness was observed in the control yoghurt. In contrast, the highest hardness was observed in the probiotic yoghurt containing 5% bael, which showed the highest level of probiotic viability. Therefore, it is apparent that there is a positive relationship between the hardness of yoghurt and probiotic viability.

Table 2. Hardness (N; means ± SD) of yoghurts during cold storage (5 °C) of 21 d

Storage Time (d)	Yoghurt formulation			
	CON	PY	PY5	PY10
1	0.053 ± 0.008$^{a, A}$	0.076 ± 0.001$^{b, A}$	0.074 ± 0.006$^{b, A}$	0.084 ± 0.002$^{b, A}$
7	0.069 ± 0.005$^{a, A}$	0.073 ± 0.001$^{ab, A}$	0.076 ± 0.008$^{ab, A}$	0.091 ± 0.008$^{b, A}$
14	0.080 ± 0.004$^{a, A}$	0.102 ± 0.014$^{ab, A}$	0.119 ± 0.007$^{b, AB}$	0.098 ± 0.004$^{ab, A}$
21	0.094 ± 0.003$^{a, AB}$	0.115 ± 0.022$^{ab, A}$	0.142 ± 0.012$^{b, AB}$	0.104 ± 0.006$^{ab, A}$

$^{a\text{-}b}$Means within a row with different superscripts were significantly different (p<0.05).
$^{A\text{-}B}$Means within a column with different superscripts were significantly different (p<0.05).
CON = Yoghurt fermented with conventional yoghurt starter culture; PY = Conventional starter culture + *Lb. rhamnosus* GG; PY5 = Conventional starter culture + *Lb. rhamnosus* GG + Bael 5%; PY10 = Conventional starter culture + *Lb. rhamnosus* GG + Bael 10%.

3.4. *Viability of Lb. rhamnosus GG*

Figure 3 shows changes in *Lb. rhamnosus* GG counts during storage of experimental yoghurts. Results from mixed ANOVA revealed that both the treatment and storage time had a significant influence on the probiotic counts (p<0.05). After 7 d of refrigerated storage, probiotic counts in bael incorporated yoghurts (PY5 and PY10) maintained at a level of >10^8 cfu/mL which was significantly higher than that of the plain probiotic yoghurt (PY) (P<0.05). Thereafter, probiotic counts in all treatments showed a gradual decline over the rest of the storage period and this decline was significant in PY and PY10 (p<0.05). Decreased viability of probiotics during cold storage is a common phenomenon observed and reported by many authors. For example, there was a gradual decrease in *Lb. acidophilus* counts in fermented buffalo milk beverages throughout 21 d of refrigerated storage [12]. Despite the declining trend in probiotic counts observed in the current study, it was maintained well above 10^7 cfu/mL throughout the storage suggesting that the buffalo milk yoghurt is an excellent dairy matrix to deliver the EPS-producing probiotic *Lb. rhamnosus* GG in sufficient quantities required to deliver therapeutic effects (10^6–10^9 cfu/mL). Perhaps, this could be due to the higher fat content on buffalo milk that preserves the viability of probiotics by increasing their resistance to acidity [50]. Previous research findings too showed that buffalo milk yoghurt preserved viability of *Lb. acidiphilus* and *Bifidobacterium bifidum* >10^7 cfu/mL over a 21 d of refrigerated storage.

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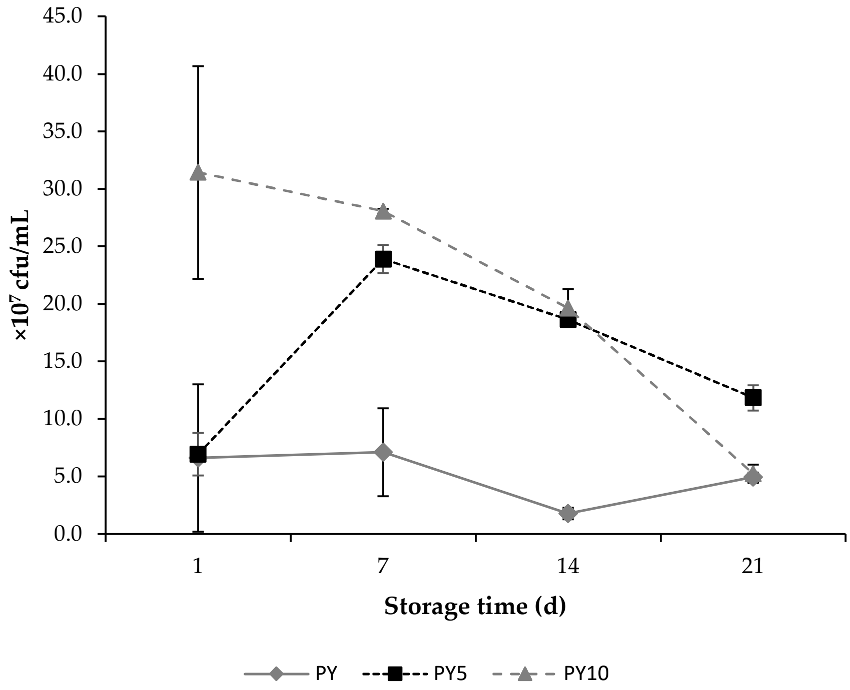

Figure 3. Viability (cfu/mL) of *Lb. rhamnosus* GG during refrigerated storage (4 °C) of buffalo milk yoghurt preparations: CON = control yoghurt produced with conventional starter cultures; PY = probiotic yoghurt containing *Lb. rhamnosus* GG; PY5 = probiotic yoghurt containing *Lb. rhamnosus* GG and 5% (w/v) bael extract; and PY10 = probiotic yoghurt containing *Lb. rhamnosus* GG and 5% (w/v) bael extract. Values are mean ± SD.

Addition of bael extract had a positive effect on the viability of *Lb. rhamnosus* GG during the refrigerated storage. This was seen after 7 and 14 d of storage where the probiotic count in bael incorporated yoghurts (PY5 and PY10) were significantly higher ($p<0.05$) compared to that of the plain probiotic yoghurt (PY) which did not contain bael. Bael pulp contains approximately 3% fiber [34,36]. Since dietary fibers can act as prebiotic substances that selectively promote the growth of certain probiotic microorganisms, the higher viable counts observed in bael extract incorporated yoghurts may be due to the prebiotic effect of these fibers. Out of the two levels of bael concentrations tested, 5% (w/v) incorporation level seems to be ideal for symbiotic product development as it maintained viable probiotic counts of $>10^8$cfu/mL even after 21 d of storage. Although 10% of bael incorporated probiotic yoghurts showed $>10^8$ cfu/mL viable counts from 1-14 d of storage, it was significantly declined after 21 d of storage. This may be due to the antimicrobial effect of phenolic compounds present in bael extract and got released during fermentation. Therefore, higher incorporation rates of bael may be detrimental to the probiotics present. Many research findings suggest that *Lb. rhamnosus* is well-survived in fermented milk products containing fruits and fruit juices such as passion fruit [28,31], mango [28,31], apricot [28], apple [31,33,51], kiwi [29,31], pineapple[29–31], peach [28,29], strawberry [29], orange [30,31], grape [31], and lemon [31].

3.5. *Sensory Properties*

The average responses of the taste panelists to the sensory attributes of different buffalo milk yoghurt formulations have been summarized in Table 3. As seen in the Table, yoghurt formulation had a significant impact on consumer liking in all attributes except for taste and aftertaste. There was no significant difference found between plain yoghurt and probiotic yoghurt for any of the sensory attribute evaluated ($p>0.05$). These results indicated that the addition of the probiotic *Lb. rhamnosus*

GG did not significantly affect sensory attributes of buffalo milk yoghurt. In other words, the sensory properties of probiotic yoghurt were comparable to that of the standard yoghurt. Similar results were reported by Hekmat and Reid [52] where they found that the sensory properties of probiotic yoghurt containing either *Lb. rhamnosus* GR-1 or *Lb. reuteri* RC-14 was comparable to that of the standard yoghurt made with conventional yoghurt starter cultures. Nevertheless, probiotic addition resulted in improved mean sensory scores for taste, aftertaste, mouthfeel and overall acceptability. It has been reported that the addition of some LAB can spoil milk and yoghurt leading to unpleasant flavor and odor [53]. Results of the current study suggested that addition of *Lb. rhamnosus* GG has not been associated with such detrimental effects in buffalo milk probiotic yoghurts.

Addition of bael extract had varying effects on sensory attributes depending on the concentration. Irrespectively of the concentration, the addition of bael extract significantly decreased mean scores for aroma ($p<0.05$). Bael fruit possesses a characteristic strong aroma which is not destroyed even after thermal processing. A total of twenty-eight (28) volatile compounds have already been identified from fully ripen bael fruit and among these, monoterpenes and sesquiterpenes are the main contributors for the characteristic aroma. On the other hand, during the extraction of bael pulp, there is a high possibility of developing off-flavors due to enzymatic activity [54]. These two factors in combination may contribute to the significantly lower mean aroma scores of bael-incorporated yoghurts (PY5 and PY10) compared to that of the control- and probiotic yoghurts. Irrespectively of the concentration, addition of bael did not significantly change mean scores for taste, aftertaste and mouthfeel attributes ($p>0.05$). Although there was no significant difference among the four treatments, formulations containing bael received considerably lower mean scores for the above sensory attributes compared to plain and probiotic yoghurts. Probiotic yoghurt containing 5% (w/v) bael, received slightly higher mean scores for appearance and color compared to probiotic yoghurt, probably due to the yellowish color resulting from added bael which may have impressed panelists. However, mean scores of appearance, color, texture and overall acceptability for probiotic yoghurt containing 10% (w/v) bael (PY10) were significantly lower than those of the other three formulations (CON, PY and PY5). These results suggested that there is a negative correlation between consumer liking and bael concentration. Similar results can be found in dahi fortified with bael in which higher sensory scores were received for the formulation containing 5% bael compared to 10% and 15% [55].

Table 3. Average responses of tasting panelists to the sensory properties of buffalo milk yoghurt preparations (like extremely = 7; neither like nor dislike = 4; dislike extremely = 1).

Characteristic	CON	PY	PY5	PY10
Appearance	5.88 ± 0.18^{a}	5.50 ± 0.26^{a}	5.79 ± 0.22^{a}	3.79 ± 0.33^{b}
Color	5.94 ± 0.15^{a}	5.65 ± 0.22^{ac}	5.74 ± 0.23^{a}	4.97 ± 0.24^{bc}
Aroma	6.03 ± 0.14^{a}	5.91 ± 0.15^{a}	4.53 ± 0.30^{b}	4.09 ± 0.28^{b}
Texture	5.59 ± 0.19^{a}	5.32 ± 0.20^{ab}	5.09 ± 0.26^{ab}	4.65 ± 0.24^{b}
Taste	5.56 ± 0.12^{a}	5.59 ± 0.16^{a}	4.85 ± 0.27^{a}	4.71 ± 0.26^{a}
After taste	5.26 ± 0.15^{a}	5.44 ± 0.19^{a}	4.82 ± 0.26^{a}	4.59 ± 0.27^{a}
Mouthfeel	5.50 ± 0.15^{a}	5.68 ± 0.15^{a}	5.00 ± 0.25^{ab}	4.74 ± 0.25^{ab}
Overall acceptability	5.56 ± 0.16^{a}	5.76 ± 0.16^{a}	5.09 ± 0.26^{a}	4.47 ± 0.25^{b}

Different superscript letters in the same row indicate a significant different ($p < 0.05$).

Values are expressed as mean ± SEM. CON = control yoghurt; PY = probiotic yoghurt containing *Lb. rhamnosus* GG; PY5 = probiotic yoghurt containing *Lb. rhamnosus* GG and 5% bael; PY10 = probiotic yoghurt containing *Lb. rhamnosus* GG and 10% bael. Values are mean ± SD.

As a whole, probiotic yoghurt received the highest mean score for overall acceptability (5.76±0.16). Moreover, there was no significant difference among the overall acceptability scores of plain yoghurt, probiotic yoghurt and yoghurt containing 5% bael. Depending on the temperature, PH and ionic strength, microbial EPS reported to have enormous functional effects in food processing which can act as viscosifiers, bio-thickeners, emulsifiers and stabilizers which in turn enhance the texture, mouthfeel and stability of the products [56]. Therefore, the high overall acceptability of the probiotic yoghurt may be due to the EPS produced by *Lb. rhamnosus* GG during the fermentation process.

4. Conclusions

The buffalo milk yoghurt matrix maintained probiotic *Lb. rhamnosus* GG counts more than 10^7 cfu/mL throughout the refrigerated storage (4 °C) of 21 d. Control yoghurt made with conventional yoghurt starter cultures showed a significant pH drop and a significantly higher level of syneresis during storage. The addition of the EPS-producing *Lb. rhamnosus* GG resulted in significantly lower post-acidification and syneresis compared to the control yoghurt. Addition of bael did not affect post-acidification, but significantly reduced the level of syneresis in probiotic yoghurt more than one third. Addition of probiotic and bael slightly increased the hardness although the increase in hardness was not significant. Addition of bael extract increased the viable probiotic count during the storage suggesting possible prebiotic effect. Out of the two bael concentrations tested (5% and 10%), 5% (w/v) incorporation level maintained viable counts of >10^8 cfu/mL at the end of 21 d storage. Addition of probiotics did not significantly affect sensory attributes of the buffalo yoghurt. Addition of bael extract, however, considerably decreased mean scores for all sensory attributes in particular aroma, taste an after taste. In summary, it can be concluded that buffalo milk yoghurt is an excellent carrier food to deliver *Lb. rhamnosus* GG which assured the delivery of viable cells at sufficient quantities to confer therapeutic effect in the host, and 5% (w/v) incorporation would be an ideal blend to develop symbiotic products.

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