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Effects of fermenting with *Lacticaseibacillus rhamnosus* GG on quality attributes and storage stability of buffalo milk yogurt incorporated with bael (*Aegle marmelos*) fruit pulp

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ABSTRACT

Background: Producing functional food by adding fruits or fruit pulps have attracted great attention. Simultaneously, buffalo milk is gaining an increasing demand as an alternative to cow milk. Thus, value addition and diversification of buffalo milk products have gained much commercial and research interest. Hence, we aimed to investigate the potentials of developing and characterizing probiotic enriched buffalo milk yogurts with bael fruit pulp using exopolysaccharides producing probiotic *Lacticaseibacillus rhamnosus* GG (LGG).
Methods: Four types of buffalo milk yogurts were tested, e.g. fermenting with the yogurt starter culture only (e.g., control) and fermenting with the combination of yogurt starter culture and LGG with varying levels (*w/v*) of bael fruit pulp incorporations, i.e., 0%, 5% and 10%. Variation in pH, syneresis, hardness, probiotic viability and sensory attributes during 21 days of storage in 4 °C were assessed for all treatments.
Results: Fermenting with LGG had a positive effect on post-acidification and syneresis rate compared to the control yogurt. Bael incorporation did not affect the post-acidification, but significantly decreased the level of syneresis at the end of storage. All probiotic formulations maintained LGG counts of $>10^7$ CFU/mL and the highest counts were observed in 5% (*w/v*) bael incorporated yogurt.
Conclusions: Results confirmed the possibility of using buffalo milk yogurt as an ideal matrix to deliver LGG with promising probiotic capacity. The use of 5% bael incorporation provides an optimal combination for synbiotic product development.

1. Introduction

There is increasing popularity for diversified buffalo milk products as novel foods compared to cow milk-derived foods, although there are not many value-added products available in the market. Up-to-date, the production of fermented buffalo milk-derived products is mainly available as traditional ethnic foods with fewer modifications [1,2]. Thus, the formulation of fermented buffalo milk products may expand the range of products with different qualities than those traditionally made with cow or buffalo milk [1,3]. Buffalo milk contains higher levels of total solids (16–19%) than cow milk, which can give better textural characteristics to yogurt [4]. 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) [5]. Greater quantities of caseins and fat from buffalo milk provide the final product with a better gel consistency and more creaminess, respectively [2]. An increasing amount of evidence suggests that buffalo milk is a suitable dairy matrix to deliver probiotics or produce synbiotic products, although incorporating probiotics into buffalo milk-derived products is challenging [1,3,5,6].

Probiotics are live microorganisms, which when administered in adequate amounts confer health benefits on the host [7]. Regular consumption of probiotics increases the relative numbers of beneficial bacteria in the colon, which in turn imposes a positive impact on immune function, digestion, metabolism, and brain-gut communication [8]. Food manufacturers are attracted to probiotics due to the projected market growth, high margins, and growing consumer demand for functional foods [9]. Lactic Acid Bacteria (LAB), mainly from the genera

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Lactobacillus, and *Bifidobacterium* have widely been utilized for the development of probiotic food [9,10]. The use of exopolysaccharide (EPS)-producing starter cultures are gaining popularity due to their water-binding and texture-promoting abilities [11]. *Lacticaseibacillus rhamnosus* GG (LGG, previously *Lactobacillus rhamnosus*) is an EPS-producing probiotic bacteria successfully employed in the manufacturing of probiotic dairy products. LGG-containing milk products reported having enormous health benefits such as prevention and/or reduction of the duration of diarrhoea, alleviation of skin conditions such as eczema and allergic reactions, enhanced immunity against dental caries, and reduced risk of urogenital infections [12]. In general, fermented buffalo milk gels produced in Sri Lanka have been reported to contain lactic acid bacteria (*Limosilactobacillus fermentum, Latilactobacillus curvatus, Lactobacillus acidophilus,* and *Lactiplantibacillus plantarum*) which can exhibit promising probiotic potentials [13]. Therefore, producing fermented buffalo milk products using buffalo milk available in Sri Lanka can deliver excellent probiotic potential.

Recently, there is a trend among food manufacturers in producing functional dairy foods containing fruit or fruit pulp [9]. 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 diarrhoea, dysentery, and peptic ulcers [14,15]. Bael fruit pulp contains approximately 0.27% fat, 1.12% protein, and 3.3% fibre in addition to many other functional and bioactive compounds including carotenoids, phenolics, alkaloids, and flavonoids [15]. The fruit pulp of bael is used to prepare delicacies such as *murabba* (a preservative made with sugar, spices, and pectin-rich fruit), puddings, and juice [14]. Comprehensive information related to the agronomical characteristics, chemical composition, and health benefits associated with bael is well documented [14–16].

It is widely accepted that at least 10^6–10^9 colony-forming units per millilitre or gram (CFU mL^{-1}or g) of viable probiotic cells must be available in the final product at the time of consumption to assure any therapeutic effect [9,10]. Probiotic bacteria grow poorly in milk. However, ingredients like sugar, vitamins, transglutaminase, whey protein concentrates, cereals, and fruits used in yogurt production improve probiotic growth by providing essential nutrients and proper conditions such as pH, and redox potential. The fat content of yogurt is another factor affecting probiotic viability [17]. Previous research showed that full-fat yogurt act as an inhibitory matrix for certain probiotic strains compared to low-fat yogurt [18]. One major limitation associated with probiotic dairy products is the loss of viability of the probiotics during cold storage. In this research, we hypothesized that the higher fat content in buffalo milk and higher dietary fibre content in beal may have a beneficial effect on probiotic viability during refrigerated storage (4 °C). Hence we aimed to determine the effects of adding bael fruit pulp on some microbiological, physicochemical, and sensory properties of buffalo milk yogurt containing EPS-producing probiotic strain *L. rhamnosus* GG.

2. Materials and methods

2.1. Preparation of bael fruit pulp

Fully ripen bael fruits were purchased from the local market. The fruit pulp was extracted after washing and de-shelling the fruits into halves. The pulp was mixed with water in a 1:1 ratio and stirred thoroughly until any visual clumps were absent. The extract was heat treated (at 60 °C for 5 min), stored in a glass container, and kept under refrigerated conditions (4 ± 1 °C) until use.

2.2. Preparation of starter cultures

Commercial freeze-dried yogurt starter culture (Yoflex®-L811) and probiotic *L. rhamnosus* GG (nu-trish® LGG®) used in the study were purchased from Chr. Hansen (Horsholm, Denmark). The yogurt starter culture contained *Streptococcus thermophilus* and *Lactobacillus delbrueckii* ssp. *bulgaricus* in 1:1 ratio. Yogurt starter and probiotic cultures were activated by inoculating 0.05% (*w/v*) with 100 mL portions in pasteurized skimmed milk on separate milk bases and incubated overnight (~18 h) at 42 °C and 37 °C, respectively. Subsequently, working cultures were prepared by inoculating skimmed milk with 5% (*v*/v) of the activated cultures.

2.3. Preparation of set-yogurt

Buffalo milk was purchased from Mahayaya Farm located in the Northwestern Province of Sri Lanka during September and October 2020. The milk base was prepared by standardising for fat content (5%) by mixing whole and 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 four treatments: (a) control yogurt manufactured with conventional yogurt culture (CON); (b) probiotic yogurt containing *L. rhamnosus* GG (PY0); (c) probiotic yogurt containing *L. rhamnosus* GG and 5% (*w/v*) bael fruit pulp (PY5); and (d) probiotic yogurt containing *L. rhamnosus* GG and 10% (w/v) bael fruit pulp (PY10). The inoculation ratios of starter cultures and bael have been mentioned in Table 1. After inoculation, the yogurt mixes were incubated at 42 °C until a pH of 4.5–4.8 was reached. Thereafter, all yogurt samples were stored under refrigerated conditions (4 ± 1 °C). During the study period, several batches of yogurts were routinely prepared following the same methodology. (See Fig. 1.)

2.4. Determination of physicochemical properties

The physicochemical properties of the yogurt products were determined at 1, 7, 14, and 21 days of refrigerated storage after production. The pH values of the milk, bael fruit pulp, and products were determined using a digital pH meter (OHAUS, STARTER 3000, US). The pH was measured just after the preparation of yogurts, after 2.5 h of fermentation, and then at 30 min intervals until the pH reaches 4.5–4.8. In addition, the pH was determined at the aforementioned weekly time points during cold storage.

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

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

The texture analysis was performed in terms of hardness using a texture analyser (Shimadzu, Kyoto, Japan) equipped with a 50 kg load cell and a piercing test jig (3 mm in diameter). Hardness was evaluated through piercing and penetration tests using yogurt, immediately after taking it from the cold storage. The test was performed directly in standard plastic containers, each filled with 70 mL of yogurt. The probe was moved at a test speed of 1 mm/s from the yogurt surface until a

Table 1
Inoculating ratios of starter cultures, probiotic culture and bael fruit pulp.

Treatment	Yogurt starter culture % (v/v)	Probiotic culture % (v/v)	Bael fruit extract % (*w/v*)
CON	4	0	0
PY0	2	2	0
PY5	2	2	5
PY10	2	2	10

Fig. 1. (A) Bael (*Aegle marmelos*) fruit and pulp, and (B) Yogurt formulations: CON = control yogurt produced with conventional yogurt starter cultures; PY0 = probiotic yogurt containing *Lb. rhamnosus* GG; PY5 = probiotic yogurt containing *Lb. rhamnosus* GG and 5% (*w/v*) bael fruit pulp; and PY10 = probiotic yogurt containing *Lb. rhamnosus* GG and 5% (w/v) bael fruit pulp.

depth of 18 mm. The force and time spent on penetration were recorded, where the average of triplicates was obtained by performing on three different surface locations within a yogurt.

2.5. Determination of probiotic viability

The viability of LGG was evaluated using the pour plate technique. One gram (1 g) of yogurt was suspended in 9 mL of 1% (*w/v*) peptone water followed by the preparation of 10-fold serial dilutions. Selective enumeration of LGG was carried out using MRS vancomycin media (pH 6.2). The media contained 50 ppm vancomycin (GUFIC®, Gujarat, India). The plates were incubated at 37 °C for 72 h under anaerobic conditions which were created using anaerobic sachets (Microbiology Anaerocult® C, Darmstadt, Germany). 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. *L. rhamnosus* GG colonies were confirmed by white, shiny, and smooth colonies approximately 2 mm in diameter [19].

2.6. Determination of sensory characteristics

Sensory evaluation of the four yogurt formulations was conducted by taste panellists aged 23–40. Panellists were recruited from the Faculty of Livestock, Fisheries and Nutrition, Wayamba University of Sri Lanka. All the panellists were regular consumers of yogurt and were selected based on interest to be volunteers for a sensory panel, time availability, non-smoking status, and lack of food allergies. Panellists evaluated four yogurt samples in individual boots under controlled lighting and temperature. During the evaluation, no verbal communication was allowed among the panellist to ensure accurate data collection. Sensory analysis was performed with yogurt products stored for 7 days under refrigerated conditions (4 ± 1 °C). They were offered to the panellists in cold conditions and presented in simultaneous multiple mode. Accordingly, forty grams (40 g) of each sample were served in uniform plastic containers labelled with a random 3-digit code. The samples were presented in a randomized order among the panellists who were instructed to rinse before tasting each sample. Each attribute (appearance, colour, 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 analyses

The whole experiment was repeated twice and the results were reported as mean ± standard deviation (SD) for the triplicate analyses. A two-way mixed ANOVA was used to determine the main effects of treatment (between-subject factor) and storage time (within-subject factor) on physicochemical parameters and probiotic viability. Means were separated using Tukey Test. Data were treated as randomized complete blocks arranged in a 4 × 4 factorial scheme (4 treatments: CON, PY0, 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 physicochemical and microbial viability data, respectively. Sensory data were analysed using the Friedman test followed by Wilcoxon signed-rank test. All statistical analyses were performed using SPSS version 22.0 (SPSS Inc., Chicago, IL) with a significance level of $P < 0.05$).

3. Results and discussion

3.1. Post acidification

The initial pH of the buffalo milk and bael fruit pulp ranged from 6.2 to 6.3 and 5.0–5.5, respectively. The addition of bael resulted in a slight reduction of pH probably due to the higher contents of oxalic, malic, and tartaric acids [14,15]. Accordingly, the average initial pH of the buffalo milk mixed with bael fruit pulp and probiotics was 6.08 ± 0.06. It took approximately 4 h of fermentation to reach the desired pH (4.5–4.8).

During storage, the growth and metabolic activity of the LAB present in the product break down carbohydrates into organic acids and the accumulation of these acids causes a reduction in pH. This phenomenon is known as post-acidification [20,21]. In the current study, the control yogurt showed a significant drop ($P < 0.05$) in pH during the storage (Fig. 2). In contrast, the decrease in pH in probiotic-containing yogurts (PY0, PY5, and PY10) was not significant ($P > 0.05$). This may be due to the buffering ability of the exopolysaccharides produced by LGG. Similar findings were reported for milk-juice beverages with fermented sheep milk and strawberry where the post-acidification was found to be significantly low in products manufactured with probiotic *Lactiplantibacillus plantarum* (previously *Lb. plantarum)* compared to those manufactured with conventional yogurt bacteria [7].

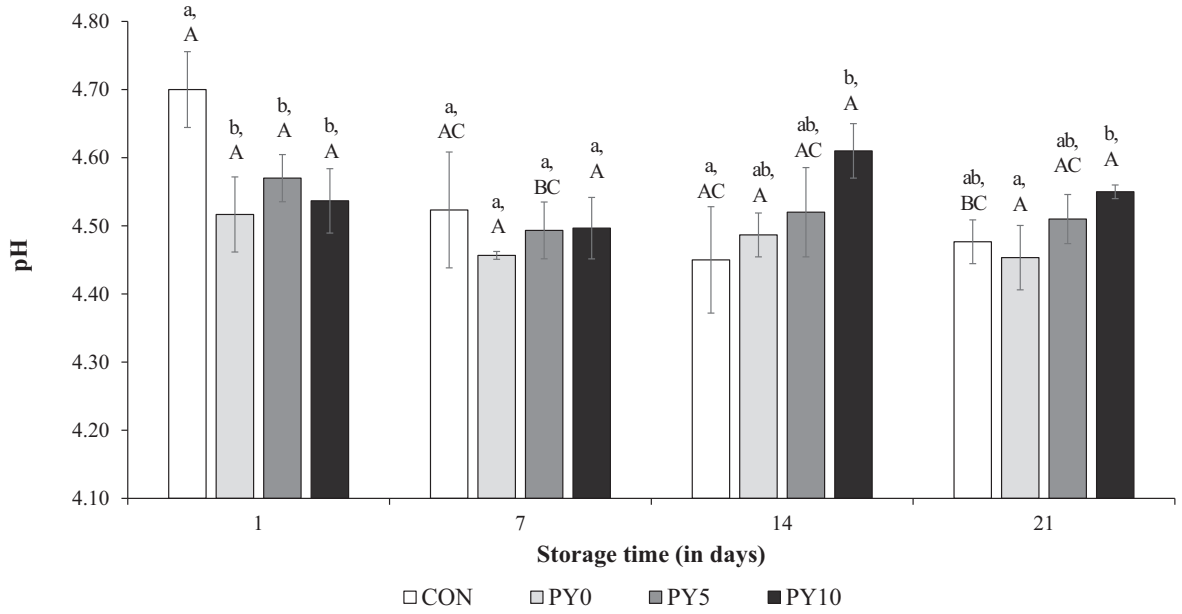

Fig. 2. Variation in pH during refrigerated storage (4 °C) of buffalo milk yogurt preparations: CON = control yogurt produced with conventional yogurt starter cultures; PY0 = probiotic yogurt containing *Lb. rhamnosus* GG; PY5 = probiotic yogurt containing *Lb. rhamnosus* GG and 5% (w/v) bael fruit pulp; and PY10 = probiotic yogurt containing *Lb. rhamnosus* GG and 10% (w/v) bael fruit pulp. Values are mean ± SD based on the average of triplicates. [a-b]Means with different lowercase letters are significantly ($P < 0.05$) different among treatments at each time point. [ABC]Means without common superscripts between time points of a particular treatment are different ($P < 0.05$).

Another interesting observation in the post-acidification profiles was that there was a hike in the pH of the probiotic yogurts on the 14th day of storage while the pH in the control yogurt was continuously decreasing. This hike was correlated with the viable probiotic counts on the 14th day as the magnitude of the pH increase was proportionate to the viable probiotic counts observed 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 PY0 which had the lowest viable probiotic count (1.77×10^7 CFU/g). Previous research showed that the pH of an acid milk curd fermented by *Lb. bulgaricus* was increasing with increasing levels of exopolysaccharides (EPS) [22]. Therefore, it is likely that the pH hike observed on the 14th day of the storage in the current study was due to the higher production of exopolysaccharides by LGG.

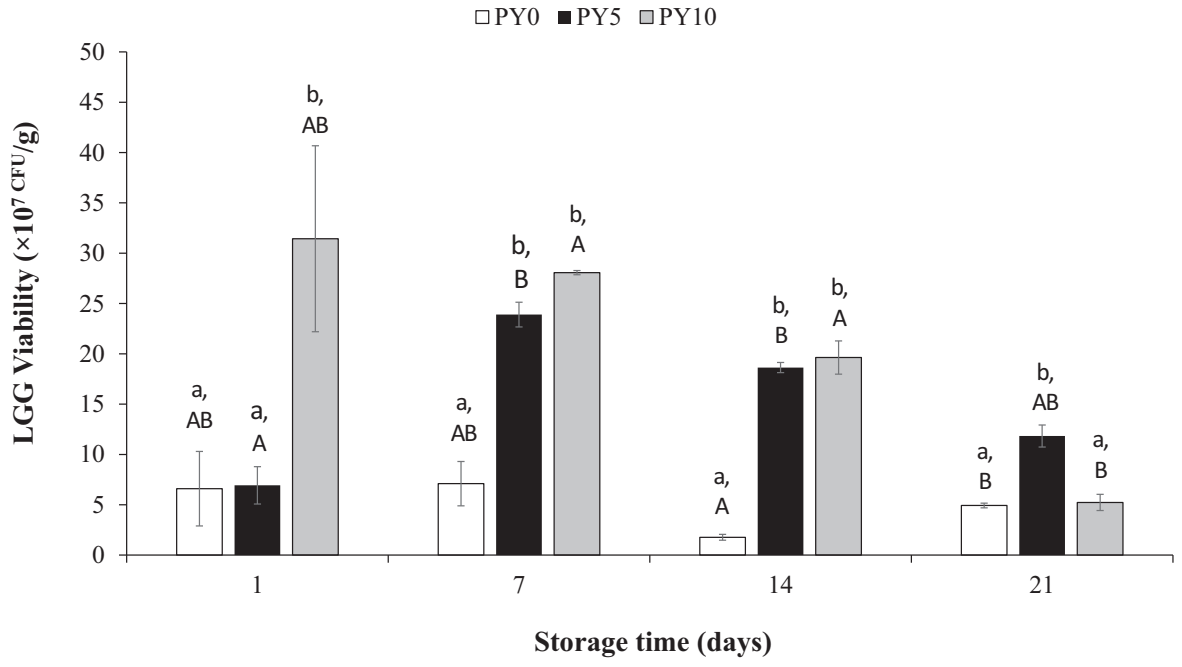

Fig. 3. Viability (CFU/mL) of *Lb. rhamnosus* GG during refrigerated storage (4 °C) of buffalo milk yogurt preparations: PY0 = probiotic yogurt containing *Lb. rhamnosus* GG; PY5 = probiotic yogurt containing *Lb. rhamnosus* GG and 5% (w/v) bael fruit pulp; and PY10 = probiotic yogurt containing *Lb. rhamnosus* GG and 10% (w/v) bael fruit pulp. Values are mean ± SD. [a-b]Means with different lowercase letters are significantly ($P < 0.05$) different among treatments at each time point. [AB]Means without common superscripts between time points of a particular treatment are different ($P < 0.05$).

Similar results were reported for acai yogurt in which a subsequent increase in pH was observed at 21 and 28 days of storage after observing a significant reduction in pH values during the first 14 days of storage [23].

Another drop in pH was observed in the probiotic yogurts after 21 days of storage. This may be due to the accumulation of organic acids produced by LGG as a result of the greater viable counts seen at the 14 days of cold storage. Another important observation of the current research is that the pH in bael-incorporated yogurts (PY5 and PY10) between 14 and 21 days of refrigerated storage was greater than those without bael (CON and PY0). Moreover, the acidity tended to increase with an increasing level of fortification. This is likely to be associated with the higher viable counts observed in bael-incorporated yogurts 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 yogurt [20], honey or apple pulp-incorporated buffalo yogurt [24], stingless bee honey-incorporated goat milk yogurt [21], and mango juice-enriched probiotic dairy drinks [9].

3.2. Viability of *L. rhamnosus GG*

Fig. 3 shows changes in *L. rhamnosus* GG counts during the storage of experimental yogurts. Results from mixed ANOVA revealed that both the treatment and storage time had a significant influence on the probiotic counts ($P < 0.05$). At day 7 of refrigerated storage, probiotic counts in bael-incorporated yogurts (PY5 and PY10) were maintained at a level of $>10^8$ CFU/g which was significantly higher than that of the plain probiotic yogurt (PY0) ($P < 0.05$). Thereafter, probiotic counts in all treatments showed a gradual decline over the rest of the storage period and this decline was significant in PY0 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 *L. acidophilus* counts in fermented buffalo milk beverages throughout 21 d of refrigerated storage [3]. Despite the declining trend in probiotic counts observed in the current study, it was maintained well above 10^7 CFU/g throughout the storage suggesting that the buffalo milk yogurt is an excellent dairy matrix to deliver the EPS-producing probiotic *L. rhamnosus* GG in sufficient quantities required to deliver therapeutic effects (10^6–10^9 CFU/mL or g). In a previous study, lipids on the matrix of buffalo Minas Frescal Cheese were reported to bind with the membranes of *Bifidobacterium* BB-12 and protect them from disruption during in vitro simulated gastrointestinal conditions [25]. This suggests that the same phenomenon may be applied to maintain probiotic viability during storage where the fat bound to the bacterial membranes may act as a protective barrier to accumulating acids in the medium.

The addition of bael extract had a positive effect on the viability of *L. rhamnosus* GG during refrigerated storage. This was seen after 7 and 14 days of storage where the probiotic count in bael-incorporated yogurts (PY5 and PY10) were significantly higher ($P < 0.05$) compared to that of the plain probiotic yogurt (PY0) which did not contain bael. Bael pulp contains approximately 3% fibre [15]. Since dietary fibre can act as prebiotic substances that selectively promote the growth of certain probiotic microorganisms, the higher viable counts observed in bael-incorporated yogurts may be due to the prebiotic effect of these fibres. Out of the two levels of bael concentrations tested, a 5% (*w/v*) incorporation level seems to be ideal for product development as it maintained viable probiotic counts of $>10^8$ CFU/g even after 21 days of storage. Although 10% of bael-incorporated probiotic yogurts showed $>10^8$ CFU/g viable counts from 1 to 14 days of storage, it significantly declined after 21 days of storage. This may be due to the antimicrobial effect of phenolic compounds present in bael extract and got released during fermentation [26–28]. Therefore, higher incorporation rates of bael may be detrimental to the probiotics present.

3.3. Syneresis

Syneresis is the expulsion of whey from the gel network and is a major quality defect in yogurts that negatively affects consumer perception [29]. Syneresis in different yogurt formulations over the refrigerated storage is depicted in Fig. 4. Statistical analysis revealed that both the treatment and storage time significantly influence syneresis in the buffalo milk yogurts ($P < 0.001$). At the 21-day of storage, the control yogurt (CON) experienced a 12% increase in syneresis ($P < 0.05$). In contrast, probiotic yogurts (PY0, PY5, and PY10) showed a 4%, 36%, and 30% decline in syneresis, respectively. After 21 days of storage, the highest syneresis value was observed for the control yogurt ($38.66 \pm 1.34\%$). A similar level of syneresis (39–42%) has previously been reported by Akgun and others [6] for buffalo milk yogurt during cold storage. Previous research findings suggest that an increase in acidity may increase syneresis in buffalo milk yogurt during cold storage [24]. Therefore, the significant increase in syneresis observed in the control yogurt may be due to the significantly higher post-acidification that occurred during storage.

Microbial EPS may be composed of either homopolysacchatrides or heteropolysaccharides and affect the texture, stability, and sensory properties of yogurt and related products. The effective concentrations of these are considerably lower compared to the commercial polysaccharides used as additives. Changes associated with EPS are caused by alterations in syneresis, viscosity, and stiffness of the fermented milk product that in turn relate to their water-binding properties and interactions with the protein network [30]. At the 21-day of refrigerated storage, the syneresis in plain probiotic yogurt (PY0) was significantly lower ($P < 0.05$) than that of the control yogurt suggesting that the addition of LGG resulted in a significantly reduced syneresis in buffalo milk yogurt. Similar results have previously been reported by Amatayakul and others [11] where the yogurts made with EPS-producing starter culture had a lower level of syneresis than yogurt produced with non-EPS-producing starter cultures. It has been reported that both the higher water-binding capacity and the modification of yogurt microstructure by EPS affect syneresis [11]. Similarly, Priyashantha and others [31] have shown probiotic starter cultures reduce the predisposition to syneresis, especially from cultures consisting of *L. rhamnosus*. Authors attributed these differences are possibly due to their ability to produce EPS, which can bind water and thus, reduce the susceptibility to expel from the protein gel network. Therefore, it is likely that the EPS produced by LGG is responsible for the reduced level of syneresis observed in probiotic yogurt in the current study.

Our results showed that the incorporation of bael significantly reduced syneresis in bael-incorporated buffalo milk yogurts leading to the lowest level of syneresis (<20%) after 21 days of cold storage. Previous literature on buffalo milk yogurt suggests that the addition of prebiotics significantly reduced syneresis by increasing the water-binding ability [1]. Therefore, it is likely that bael extract exerts a potential prebiotic effect which in turn resulted in significantly low levels of syneresis in bael-incorporated yogurt. Moreover, the level of syneresis in the 5% bael – incorporated and 10% bael-incorporated yogurts 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 [6]. There was another significant drop in syneresis in bael-incorporated yogurts (PY5 & PY10) at 21 days of storage compared to that at 14 days. This may also be due to the rearrangement of the gel network.

3.4. Hardness

The texture is a key determinant of yogurt quality and affects appearance, mouthfeel, and overall acceptability [32]. Hardness is the

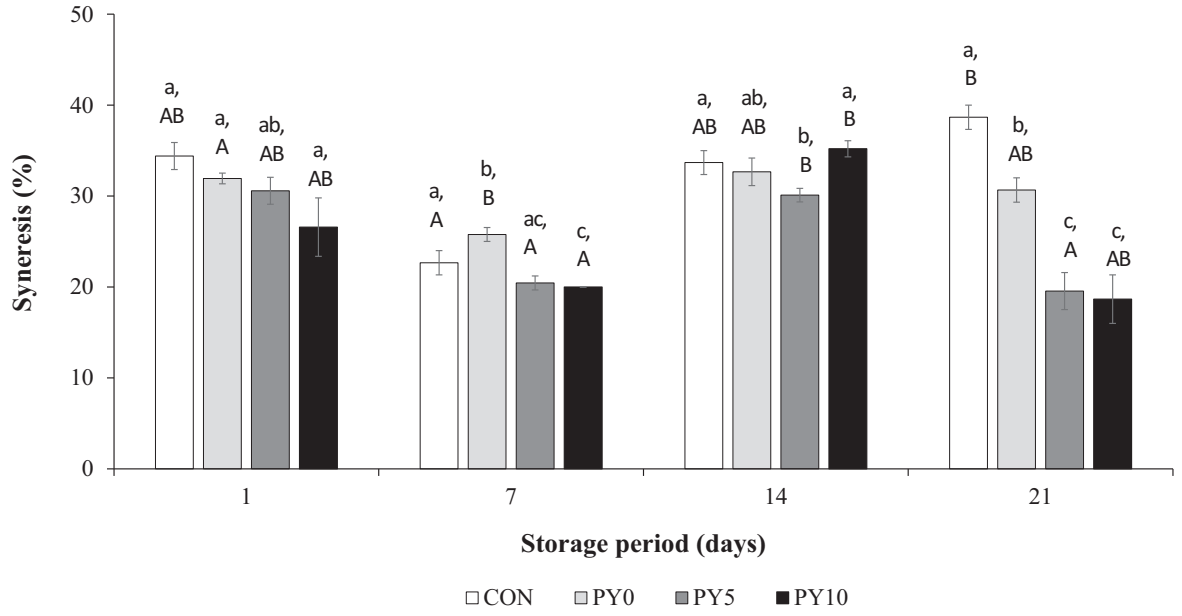

Fig. 4. Syneresis changes during refrigerated storage (4 °C) of buffalo milk yogurt preparations: CON = control yogurt produced with conventional yogurt starter cultures; PY0 = probiotic yogurt containing *Lb. rhamnosus* GG; PY5 = probiotic yogurt containing *Lb. rhamnosus* GG and 5% (w/v) bael fruit pulp; and PY10 = probiotic yogurt containing *Lb. rhamnosus* GG and 10% (w/v) bael fruit pulp. Values are mean ± SD. [a-b]Means with different lowercase letters are significantly ($P < 0.05$) different among treatments at each time point. [AB]Means without common superscripts between time points of a particular treatment are different ($P < 0.05$).

most important parameter for the evaluation of yogurt texture and is also considered a measure of yogurt firmness [33]. The hardness values for different yogurt formulations during 21 days of storage 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 and others [34] in cow milk yogurt stored for 28 days. One interesting observation throughout the storage was that the hardness values for probiotic yogurts (PY0, PY5, and PY10) were relatively higher than the control yogurt. In a previous study, LGG reported producing EPS which could improve the texture of yogurt by interacting with the free water in the gel structure [34]. In another study, the firmness of yogurts made using capsular EPS- or ropy EPS-producing starter cultures was generally lower than that in yogurts produced with non-EPS-producing starter cultures [11]. These observations suggest that EPS produced by EPS-producing probiotic strains could increase the hardness of yogurt during storage. Therefore, it seems that the EPS produced by LGG was responsible for the higher hardness observed among the probiotic yogurts.

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

Storage time (d)	Yogurt formulation			
	CON	PY0	PY5	PY10
1	0.053 ± 0.008$^{a, AB}$	0.076 ± 0.001$^{b, A}$	0.074 ± 0.006$^{b, AC}$	0.084 ± 0.002$^{b, A}$
7	0.069 ± 0.005$^{a, A}$	0.073 ± 0.001$^{ab, A}$	0.076 ± 0.008$^{ab, BC}$	0.091 ± 0.008$^{b, A}$
14	0.080 ± 0.004$^{a, AB}$	0.102 ± 0.014$^{ab, A}$	0.119 ± 0.007$^{b, A}$	0.098 ± 0.004$^{ab, A}$
21	0.094 ± 0.003$^{a, B}$	0.115 ± 0.022$^{ab, A}$	0.142 ± 0.012$^{b, A}$	0.104 ± 0.006$^{ab, A}$

[a-b] Means within a row with different superscripts were significantly different ($P < 0.05$).
[A-C]Means within a column with different superscripts were significantly different ($P < 0.05$).
CON = Yogurt fermented with conventional yogurt starter culture; PY0 = Conventional starter culture + *Lb. rhamnosus* GG; PY5 = Conventional starter culture + *Lb. rhamnosus* GG + Bael 5%; PY10 = Conventional starter culture + *Lb. rhamnosus* GG + Bael 10%.

3.5. Sensory properties

The average responses of the taste panellists to the sensory attributes of different buffalo milk yogurt formulations have been summarized in Table 3. Yogurt formulation had a significant impact on consumer liking in all attributes except for taste and aftertaste. There was no significant difference between plain yogurt and probiotic yogurt for any of the sensory attributes tested ($P > 0.05$). These results indicated that the addition of the probiotic *L. rhamnosus* GG did not significantly affect the

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

Characteristic	CON	PY0	PY5	PY10
Appearance	5.88 ± 0.18^{a}	5.50 ± 0.26^{a}	5.79 ± 0.22^{a}	3.79 ± 0.33^{b}
Colour	5.94 ± 0.15^{a}	5.65 ± 0.22^{a}	4.97 ± 0.24^{b}	5.74 ± 0.23^{a}
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 yogurt; PY0 = probiotic yogurt containing *Lb. rhamnosus* GG; PY5 = probiotic yogurt containing *Lb. rhamnosus* GG and 5% bael; PY10 = probiotic yogurt containing *Lb. rhamnosus* GG and 10% bael.

sensory attributes of buffalo milk yogurt. In other words, the sensory properties of probiotic yogurt were comparable to that of standard yogurt. Similar results were reported by Hekmat and Reid [35] where they found that the sensory properties of probiotic yogurt containing either *L. rhamnosus* GR-1 or *Lactobacillus reuteri* RC-14 were comparable to that of the standard yogurt made with conventional yogurt starter cultures. Nevertheless, in the present study, probiotic addition resulted in improved mean sensory scores for taste, aftertaste, mouthfeel, and overall acceptability. Variables such as the composition of milk from distinct species, lactic bacterium strain, and yogurt formulation and production conditions yield diverse fermentation metabolites profiles. This may be the reason for the above observation as Hekmat and Reid had tested a yogurt prepared with cow milk but not with buffalo milk. Probiotic strains used (*Lactobacillus rhamnosus* GR-1 and *Lactobacillus reuteri* RC-14) is also different in the two studies.

The addition of bael fruit had varying effects on sensory attributes. Irrespectively of the concentration, the addition of bael significantly decreased mean scores for aroma ($P < 0.05$). Bael fruit possesses a characteristic aroma resulting from the volatile compounds present and is not destroyed even after thermal processing [36]. Perhaps this may be contributed to the significantly lower mean aroma scores of bael-incorporated yogurts (PY5 and PY10) compared to that of the control- and probiotic yogurts. Irrespective of the concentration, the 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 (CON) and plain probiotic (PY0) yogurts. Probiotic yogurt containing 5% (*w/v*) bael, received slightly higher mean scores for appearance compared to probiotic yogurt, probably due to the yellowish colour resulting from added bael which may have impressed panellists. However, mean scores of appearance, texture, and overall acceptability for probiotic yogurt containing 10% (w/v) bael (PY10) were significantly lower than those of the other three formulations (CON, PY0, and PY5). These results suggested that there is a negative correlation between consumer liking and bael concentration. Similar results can be found in *dahi* (a curd of soured curdled milk that is popular in India) fortified with bael in which higher sensory scores were received for the formulation containing 5% bael compared to 10% and 15% [37].

As a whole, probiotic yogurt 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 yogurt (CON), plain probiotic yogurt (PY0), and yogurt containing 5% bael (PY5). Depending on the temperature, pH, and ionic strength, microbial EPS reported having 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 [38]. Therefore, the high overall acceptability of the probiotic yogurt may be due to the EPS produced by *Lb. rhamnosus* GG during the fermentation process.

4. Conclusions

In summary, we concluded that buffalo milk yogurt is an excellent matrix to deliver *L. rhamnosus* GG (LGG) which assured the delivery of viable cells at sufficient quantities (more than the minimum therapeutic dose of 10^6 CFU/mL or CFU/g) to confer therapeutic effect in the host. The addition of LGG and beal-fruit pulp do not alter the acidity/pH of the respective products over the storage period. Bael-fruit extract slightly increases the pH/acidity of the product. LGG significantly reduces the degree of syneresis in buffalo milk yogurt and bael-fruit extract further reduces syneresis when combined with LGG. Both concentrations of bael-fruit extract enhance probiotic viability up to 14 days of storage. Only 5% of bael-fruit extract significantly maintains probiotic counts until 21 days of refrigerated storage suggesting that 5% (*w/v*) incorporation would be an ideal blend to ensure higher probiotic survivability. LGG does not alter the sensory properties of plain buffalo yogurt. The incorporation of beal-fruit extract negatively affects most of the sensory attributes, particularly taste, and aroma, but positively affects colour and appearance.

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Ethical statement

No animals or humans were used in experimental design or the study.

CRediT authorship contribution statement

Dinusha Yapa: Conceptualization, Methodology, Software, Validation, Formal analysis, Investigation, Data curation, Writing – original draft, Visualization. **D.M.D. Rasika:** Conceptualization, Methodology, Software, Validation, Resources, Writing – review & editing, Visualization, Supervision, Project administration. **W.A.D.V. Weerathilake:** Conceptualization, Methodology, Software, Validation, Resources, Writing – review & editing, Visualization, Supervision. **Jithmi Siriwardhana:** Conceptualization, Methodology, Software, Validation, Resources, Writing – review & editing, Visualization, Supervision. **Hasitha Priyashantha:** Conceptualization, Methodology, Software, Validation, Resources, Writing – review & editing, Visualization, Supervision.

Declaration of Competing Interest

The authors declare no conflict of interest.

Data availability

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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