Microbial and Sensory Analysis of Soy and Cow Milk-Based Yogurt as a Probiotic Matrix for *Lactobacillus rhamnosus* GR-1

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Abstract: Plant-based milk alternatives represent a growing sector of the functional food industry due to consumer demand for more nutritious and sustainable options. Soymilk is abundant in fibre, phytosterols, and isoflavones. In contrast, cow milk has a high cholesterol and caloric content, superior organoleptic characteristics, and a well-established probiotic delivery matrix. Supplementing cow milk with soymilk to produce probiotic yogurt may enhance the nutritional value, sensory profile, and probiotic delivery capacity of the final product. In order to investigate the probiotic potential and sensory appeal of this blend, four yogurt mixtures were prepared by incorporating 0% (T1), 25% (T2), 50% (T3), or 75% (T4) soymilk in cow milk. The viability of *Lactobacillus rhamnosus* GR-1 and pH were evaluated during fermentation (6 h) and refrigerated storage (30 days). Additionally, consumer acceptability was determined through a sensory evaluation. *L. rhamnosus* GR-1 reached viable counts of $10^8$ colony forming units (CFU)/mL in all treatments. Sensory panellists provided higher hedonic scores to T1 for appearance and texture compared to T2–T4, but flavour and overall acceptability ratings amongst T1–T4 were comparable. These results serve as an indication for the successful fortification of cow and soymilk yogurt mixtures with *L. rhamnosus* GR-1.

Keywords: probiotic yogurt; *Lactobacillus rhamnosus* GR-1; soymilk; sensory evaluation

1. Introduction

The consumption of soy-based foods, such as soymilk, tofu, and tempeh, has soared immensely due to heightened awareness of their composition of micronutrients (i.e., B complex vitamins, vitamin K, magnesium, phosphorus), unsaturated fatty acids, dietary fibre, protein, phytosterols, and isoflavones [1–3]. Additionally, the adoption of plant-based diets in recent years, such as vegetarianism and veganism, for various health, ethical, religious and/or cultural considerations also played a pivotal role in promoting the increased consumption of soy [1–3].

Soybeans have been investigated extensively for their role in mitigating symptoms associated with menopause, hyperlipidaemia, and the risk for various chronic diseases, such as breast cancer and cardiovascular disease (CVD), upon observation of a lower prevalence of these conditions amongst certain cultures wherein soy represents a substantial component of the diet [1,2]. For instance, the saponin, fibre, and lecithin components of soy are proposed to exert a hypocholesterolemic effect, which helps minimise CVD-related mortality risk [1,2]. Moreover, soymilk, the aqueous extract of soy, has been disclosed as an acceptable substrate for the growth of lactic acid bacteria due to its oligosaccharide, amino acid, and peptide constituents in numerous studies, thereby indicating probiotic carrier potential [4–7]. Previous studies also delved into the plethora of benefits that soy-based foods derive from the process of fermentation [4–7]. For instance, De Boever et al. supplemented
Fermentation with soygerm powder and incubated the solution with *Lactobacillus reuteri* [8]. The lipid, saponin, and lecithin constituents of the soygerm powder were postulated to preserve viable counts of *L. reuteri* observed in the study, even when exposed to deleterious bile salts [8]. In turn, *L. reuteri* promoted conversion of glycosides to bioactive aglycones through its β-glucosidase activity [3,9]. Similarly, Marazza et al. observed an improvement in isoflavone bioavailability through fermentation of soymilk with *L. rhamnosus* CRL981 [9]. The fermented soy beverage exhibited better antioxidant potential, nutritional value, and health-promoting effects compared to the unfermented soy [9]. In addition to promoting the functional properties, fermentation of soy-based foods has been demonstrated to enhance the flavour profile, texture, anti-nutritional constituents (i.e., trypsin inhibitors, phytic acid, and saponin), mineral bioavailability (i.e., zinc, calcium) and digestibility [2,10–12]. Fermentation, particularly with mixed cultures, may also improve the protein content, solubility, and availability of free amino acids [13,14].

Viable microorganisms which confer health benefits upon a host when present in adequate amounts are termed probiotics [15]. A minimum level of 10^6 colony forming units of probiotic bacteria per millilitre (CFU/mL) of the carrier food product are recommended at the time of consumption to claim probiotic potency for optimum therapeutic benefits [16].

*Lactobacillus rhamnosus* GR-1 is a facultative anaerobe with probiotic potential, which was used in the production of the first probiotic fermented milk in Africa [17]. Since then, it has been employed for numerous humanitarian-driven projects in Africa targeted towards individuals living with poverty, malnutrition, and infectious diseases, such as HIV and malaria, and represents the second most extensively studied strain of the *L. rhamnosus* species [7,17]. Its applications to human health arise from its ability to survive transit and colonise the gastrointestinal and urogenital tracts [17,18]. These health benefits range from alleviating intestinal microflora dysfunction, reducing skin rashes, and improving gastrointestinal distress, to enhancing immunity and combatting respiratory tract diseases [17,18]. *L. rhamnosus* GR-1 has also been demonstrated to reduce the incidence of fungal and bacterial infections of the urogenital tract, particularly in collaboration with *L. reuteri* RD-14, by more than 50% in one year [17–19]. The safety profile of *L. rhamnosus* GR-1 was established through its application in previous studies with immunocompromised and/or malnourished patients with HIV and inflammatory bowel diseases [17]. For instance, Reid and Hekmat explored the effect of probiotic yogurt fortified with *L. rhamnosus* GR-1 as an adjunct to the diets of patients living with HIV and AIDS on their productivity, HIV symptoms, and nutrient intake [17]. The preliminary results indicated improvements in all three primary outcome measures without any harmful side effects [17].

Traditionally, fermented, dairy-based matrices are used as agents for probiotic delivery, such as yogurt and cheese [20]. In recent years, due to the growing interest in health eating and sustainable food production practices, plant-based media derived from cereals, fruits, vegetables, and legumes have also been subjected to investigation for their ability to serve as carriers for probiotics as they represent a huge growth potential for the functional food market [20,21]. However, despite the growing demand for plant-based milk alternatives, many consumers still prefer cow milk due to its status as a staple food in North America, nutrient content, habitual consumption, better sensory attributes, and health benefits, such as calcium content and promotion of bone and dental health [22,23].

Consequently, this investigation was undertaken to elucidate the influence of soy and cow milk-based yogurt blends on the viability of *L. rhamnosus* GR-1 over 6 h of fermentation and 30 days of refrigerated storage. Additionally, the sensory appeal of these products amongst consumers was evaluated to ensure probiotic fortification does not adversely impact the organoleptic properties of either beverage.
2. Materials and Methods

2.1. MRS Broth and Probiotic Stock Solution Preparation

de Man, Rogosa, and Sharpe (MRS) (EMD Millipore Corporation, Gibbstown, NJ, USA) broth was prepared at a 5.22% weight per volume (w/v) ratio in distilled water. The solution was distributed into 20 mL test tubes and autoclaved at 121 °C for 15 min. Following sterilisation, the MRS broth was refrigerated at 4 °C overnight. *L. rhamnosus* GR-1 (10% w/v) (Canadian Centre for Human Microbiome and Probiotic Research, Lawson Health Research Institute, London, ON, Canada) was inoculated in the MRS broth under aseptic conditions, incubated anaerobically at 37 °C for 24 h using a GasPak anaerobic system (BD GasPak™ EZ Container System, Becton Dickinson & Co., Sparks, BD, USA), and stored under refrigerated conditions at 4 °C. The preparation of probiotic stock solution in MRS broth was conducted routinely at 10-day intervals to preserve viable counts of *L. rhamnosus* GR-1 and to allow for proliferation.

2.2. Probiotic Mother Culture Preparation

Partially skimmed cow milk (1% milk fat) (Sealtest® Partly Skimmed Milk, Agropur Dairy Cooperative, Saint-Hubert, QC, Canada), was purchased from Sobeys, London, ON, Canada, covered with aluminium foil, and autoclaved at 121 °C for 15 min. Following sterilisation, the temperature was gradually reduced to 35 °C in a water bath. Subsequently, 2% (w/v) probiotic stock solution was inoculated in the milk, gently stirred to ensure equal distribution of the inoculum, and incubated anaerobically at 38 °C for 6 h using a GasPak anaerobic system (BD GasPak™ EZ Container System, Becton Dickinson & Co., Sparks, BD, USA).

Fermentation and Storage of Probiotic Yogurt

Partially skimmed cow milk (1% milk fat) (Sealtest® Partly Skimmed Milk, Agropur Dairy Cooperative, Saint-Hubert, QC, Canada) and plain, unsweetened soymilk (Earth’s Own Organic So Nice™ Original Soy, Vancouver, BC, Canada) were purchased from Sobeys in London, ON, Canada. Milk treatments were prepared in the following proportions: Treatment 1 contained 100% (w/v) cow milk (control), Treatment 2 contained 75% (w/v) cow milk and 25% (w/v) soymilk, Treatment 3 contained 50% (w/v) cow milk and 50% (w/v) soymilk, and Treatment 4 contained 20% (w/v) cow milk and 75% (w/v) soymilk.

Sucrose (5% w/v) was added to all treatments to improve the sensory profile and they were stirred prior to pasteurisation. Each treatment was heated in a water bath, held at a temperature of 85–87 °C for 30 min, and cooled to 40 °C. Thereafter, 3% (w/v) plain yogurt starter culture (2% milk fat) (Astro® Original Balkan Yogourt, Plain, No Gelatin, Parmalat Canada Inc., Toronto, ON, Canada) from Sobeys, London, ON, Canada and 4% (w/v) probiotic mother culture were inoculated in each sample under aseptic conditions and stirred well. This probiotic culture concentration has been used in previous studies [24,25]. Each treatment was distributed into eight, 30 mL beakers. Beakers 1–4 represented the four fermentation timepoints (0, 2, 4, and 6 h) and beakers 5–8 represented the four refrigerated storage timepoints (1, 10, 20, and 30 days). All beakers were covered using aluminium foil.

Microbial counts and pH measurements were performed every two hours over a total of 6 h of fermentation, and every ten days over 30 days of cold storage. Beaker 1 for each treatment served as the control and it was set aside. Beakers 2, 3, and 4 were incubated at 38 °C for 2, 4, and 6 h, respectively. Following fermentation, these treatments did not undergo refrigeration and were discarded. Beakers 5, 6, 7, and 8 were incubated at 38 °C for 6 h and stored at 4 °C under refrigerated conditions for 1, 10, 20, and 30 days, respectively. All treatments were subjected to two true replications under the same experimental conditions to account for any variability between the results for microbial and pH analyses.
2.3. Microbial Analysis

An enumeration of viable colonies of *L. rhamnosus* GR-1 was conducted for all treatments following the four fermentation (0, 2, 4, and 6 h) and refrigerated storage (1, 10, 20, and 30 days) timepoints. Each sample (11% *w/v*) was subjected to serial, 10-fold dilutions in sterile saline solution (0.85% *w/v*) to produce five dilution factors: $10^{-1}$, $10^{-2}$, $10^{-3}$, $10^{-6}$, and $10^{-7}$. 0.1 mL aliquots of each sample were transferred to two selective MRS agar plates per dilution factor. These MRS plates were prepared using 5.22% (*w/v*) MRS (EMD Millipore Corporation, Gibbstown, NJ, USA), 1.5% (*w/v*) agar (EMD Millipore Corporation, Gibbstown, NJ, USA), and 0.0015% (*w/v*) fusidic acid (Enzo Life Sciences, Farmingdale, NY, USA). The plates were incubated anaerobically at 37 °C for 24 h using a GasPak anaerobic system (BD GasPak™ EZ Container System, Becton Dickinson & Co., Sparks, BD, USA). Following incubation, bacterial colonies were recorded in the form of CFU/mL. An average microbial count in CFU/mL was determined for the two replications for each sample.

2.4. pH Analysis

pH was measured for all treatments following the four fermentation (0, 2, 4, and 6 h) and refrigerated storage (1, 10, 20, and 30 days) timepoints by means of a calibrated pH meter (VWR® Symphony™ B10 pH Meter, VMR International, Radnor, PA, USA). An average value for the pH was determined for each sample through the two true replications of the experiment.

2.5. Sensory Evaluation

One hundred and twenty untrained panellists, consisting of seven males and 113 females between the ages of 18–55 years, participated in a sensory evaluation held at the Sensory Lab of the Academic Pavilion at Brescia University College, London, ON, Canada. Nine invalid and/or incomplete responses were excluded, bringing the total count to 111 responses. All interested participants were required to provide written consent and were excluded if they were allergic and/or intolerant to soy and/or cow milk, unable to provide consent, pregnant, and/or undergoing chemotherapy. Additionally, participants who had diabetes, required a translator, or those who did not satisfy the age requirement were also excluded. Approval for the sensory evaluation was granted by the Health Sciences Research Ethics Board at Western University.

Eligible panellists were guided to well-lit sensory booths and provided with trays, which consisted of four portion cups representing each of the four treatments, a CAD 10 Tim Horton’s gift card as an honorarium, and a sensory evaluation questionnaire. Treatments 1–4 were prepared as detailed in Section 2.2, incubated at 38 °C for 6 h, and refrigerated at 4 °C for four days. All treatments were stirred prior to serving to obtain the drinkable format of a yogurt beverage and portioned in uniform, 30 mL disposable cups, which did not impart any flavour to the treatments and were large enough to allow for retasting.

Participants were instructed to limit contact with their neighbours, taste the treatments from left to right, and to cleanse the palette with water between tastings to eliminate any residual flavours. Participants indicated their ratings for the appearance, flavour, texture, and overall acceptability for each sample using a nine-point hedonic scale ranging from 1 (dislike extremely) to 9 (like extremely). Participants were also asked to indicate the sample(s) they are most and least likely to purchase, their consumption patterns of probiotic yogurt, and their views about incorporating soymilk in yogurt.

2.6. Statistical Analysis

Statistical analyses were conducted using IBM SPSS Statistics 25.0 (IBM Corporation, Armonk, NY, USA). The data expressed are average values from the two replicate determinations and are presented in the form of mean ± standard deviation (SD). A one-way repeated measures analysis of variance (RMANOVA) was used to analyse the effect of fermentation and refrigerated storage on viability of *L. rhamnosus* GR-1 and pH between and within treatments over time, as well as differences in mean
hedonic scale ratings for appearance, flavour, texture, and overall acceptability between treatments for all participants. A post-hoc Fisher’s least significance difference (LSD) test was used to conduct pair-wise comparisons of means when a significance ($p < 0.05$) was detected.

3. Results

3.1. Microbial Analysis

A minimum of $10^6$ CFU/mL viable bacterial colonies should be present in a food product at the time of consumption for it to confer therapeutic benefits upon the host [16]. *L. rhamnosus* GR-1 reached mean viable counts of at least $10^8$ CFU/mL in all treatments at all fermentation timepoints (Table 1). The mean counts ($\times 10^8$ CFU/mL) of *L. rhamnosus* GR-1 in the unfermented treatments were 2.00 ($\pm 1.53$), 5.60 ($\pm 6.64$), 4.56 ($\pm 5.19$), and 2.30 ($\pm 1.25$) for Treatments 1–4, respectively (Table 1). After 6 h of fermentation, the mean microbial counts were 3.79 ($\pm 3.54$), 5.29 ($\pm 2.93$), 8.59 ($\pm 7.38$), and 5.75 ($\pm 4.64$) in Treatments 1–4, respectively (Table 1). This change in microbial counts over the duration of fermentation was not statistically significant ($p > 0.05$) for any treatment. When comparing the differences in mean microbial counts between treatments at each fermentation timepoint, viability of *L. rhamnosus* GR-1 in the soy-based treatments (i.e., Treatments 2–4) was comparable to that of the control (i.e., Treatment 1). There were no statistically significant differences ($p > 0.05$) in mean counts between treatment types. These results indicate the addition of soymilk to produce probiotic yogurt and the duration of fermentation did not adversely impact the growth, viability, or survival of *L. rhamnosus* GR-1 during fermentation.

| Treatment | Mean Counts ($\times 10^8$ CFU/mL) of *L. rhamnosus* GR-1 | p-Value |
|-----------|----------------------------------------------------------|---------|
| 1         | 2.00 ± 1.53, 2.55 ± 1.23, 4.75 ± 4.06, 3.79 ± 3.54      | 0.37    |
| 2         | 5.60 ± 6.64, 5.05 ± 3.07, 2.26 ± 1.07, 5.29 ± 2.93      | 0.43    |
| 3         | 4.56 ± 5.19, 4.49 ± 4.12, 6.81 ± 5.55, 8.59 ± 7.38      | 0.57    |
| 4         | 2.30 ± 1.25, 2.78 ± 1.79, 3.29 ± 2.05, 5.75 ± 4.64      | 0.16    |

A one-way repeated-measures ANOVA was used to analyse statistically significant differences ($p < 0.05$) between mean microbial counts over 6 h of fermentation in four probiotic yogurt treatments. (CFU indicates colony forming units; Treatment 1, 100% cow milk (control); Treatment 2, 75% cow milk + 25% soymilk; Treatment 3, 50% cow milk + 50% soymilk; Treatment 4, 25% cow milk + 75% soymilk).

The mean viable count of $10^8$ CFU/mL was maintained over the 30-day refrigerated storage in all treatments (Table 2). On day 1 of refrigerated storage, mean microbial counts were 7.64 ($\pm 5.96$), 6.97 ($\pm 5.35$), 14.11 ($\pm 13.50$), and 12.11 ($\pm 19.25$) for Treatments 1–4, respectively (Table 2). After 30 days of refrigerated storage, mean microbial counts were 5.11 ($\pm 4.17$), 5.79 ($\pm 1.76$), 8.09 ($\pm 4.08$), and 7.86 ($\pm 4.14$) for Treatments 1–4, respectively (Table 2). This change in microbial counts from day 1 to day 30 of storage was not statistically significant ($p > 0.05$) for any treatment (Table 2). Additionally, the mean microbial counts were comparable between treatments at each timepoint without any statistically significant differences ($p > 0.05$) between them (Table 2). Thus, these results also demonstrate the addition of soymilk to produce probiotic yogurt did not adversely impact the growth, viability, survival, and shelf life of *L. rhamnosus* GR-1-supplemented probiotic yogurt.
Table 2. Viable counts (Mean ± SD) of Lactobacillus rhamnosus GR-1 in probiotic yogurt treatments following 1, 10, 20, and 30 days of refrigerated storage.

| Treatment | 1 day     | 10 days   | 20 days   | 30 days   | p-Value |
|-----------|-----------|-----------|-----------|-----------|---------|
| 1         | 7.64 ± 5.96 | 5.78 ± 4.82 | 4.51 ± 2.29 | 5.11 ± 4.17 | 0.68    |
| 2         | 6.97 ± 5.33 | 14.80 ± 11.80 | 6.67 ± 2.55 | 5.79 ± 1.76 | 0.11    |
| 3         | 14.11 ± 13.50 | 14.11 ± 16.40 | 9.88 ± 8.38 | 8.09 ± 4.08 | 0.75    |
| 4         | 12.11 ± 19.25 | 10.5 ± 9.05 | 10.80 ± 8.94 | 7.86 ± 4.14 | 0.93    |

A one-way repeated-measures ANOVA was used to analyse statistically significant differences (p < 0.05) between mean microbial counts over 30 days of refrigerated storage in four probiotic yogurt treatments. (CFU indicates colony forming units; Treatment 1, 100% cow milk (control); Treatment 2, 75% cow milk + 25% soymilk; Treatment 3, 50% cow milk + 50% soymilk; Treatment 4, 25% cow milk + 75% soymilk).

3.2. pH Analysis

pH analysis was conducted during 6 h of fermentation and 30 days of refrigerated storage. At each fermentation timepoint, statistically significant (p < 0.05) differences in mean pH between the four treatment types were not observed, which indicates the proportion of soymilk in these treatments may not have been responsible for the observed changes, but rather the length of fermentation. However, significant differences (p < 0.05) in mean pH during fermentation were detected within each treatment, except for Treatment 1, thus pairwise comparisons were conducted.

Treatments 2–4 experienced an increase in acidity during fermentation, which may be explained by the lower buffering capacity of soymilk compared to cow milk [4,6]. This rise in acidity was more pronounced in the first 2 h of fermentation for each sample. For instance, the mean initial pH of Treatment 2 was 5.85 (±0.07), which was significantly higher (p < 0.05) than 5.10 (±0.28), 4.60 (±0.14), and 4.35 (±0.07), the pH values after 2, 4, and 6 h of fermentation (Table 3). Following 2 h of fermentation of each sample, the decline in pH was more gradual, with significant changes in pH being observed at fewer intervals during fermentation. For example, the pH of Treatment 4 after 2 h of fermentation (4.70 ± 0.28) was significantly higher (p < 0.05) than that after 6 h of fermentation (4.25 ± 0.07). Between 2–6 h of fermentation, pH remained fairly stable (Table 3).

Table 3. Mean pH (Mean ± SD) of probiotic yogurt treatments containing Lactobacillus rhamnosus GR-1 following 0, 2, 4, and 6 h of fermentation.

| Treatment | 0 h       | 2 h       | 4 h       | 6 h       | p-Value |
|-----------|-----------|-----------|-----------|-----------|---------|
| 1         | 5.95 ± 0.21 | 5.25 ± 0.49 | 4.75 ± 0.07 | 4.40 ± 0.28 | 0.053   |
| 2         | 5.85 ± 0.07 a | 5.10 ± 0.28 b | 4.60 ± 0.14 b,c | 4.35 ± 0.07 c | 0.013   |
| 3         | 5.50 ± 0.14 a | 4.80 ± 0.00 b | 4.25 ± 0.07 c | 4.15 ± 0.07 c | 0.002   |
| 4         | 5.35 ± 0.21 a | 4.70 ± 0.28 b | 4.40 ± 0.28 b,c | 4.25 ± 0.07 c | 0.005   |

A one-way repeated-measures ANOVA and Fisher’s least significant difference test were used to analyse statistically significant differences (p < 0.05) between mean pH values of four probiotic yogurt treatments over 6 h of fermentation. In a row, mean pH values for each treatment followed by the same superscript letter (a–c) are not significantly different (p > 0.05). (Treatment 1 indicates 100% cow milk (control); Treatment 2, 75% cow milk + 25% soymilk; Treatment 3, 50% cow milk + 50% soymilk; Treatment 4, 25% cow milk + 75% soymilk).

Over 30 days of refrigerated storage, statistically significant (p < 0.05) differences in mean pH between the four treatment types were not observed. However, significant differences (p < 0.05) in mean pH were detected within Treatments 2 and 4. The pH of Treatment 2 declined significantly (p < 0.05) throughout the storage period (Table 4). The mean pH on day 1 was 4.20 (±0.00), which reduced significantly (p < 0.05) to 4.10 (±0.02), 4.02 (±0.02), and 3.97 (±0.05) on days 10, 20, and 30, respectively (Table 4). Similarly, the mean initial pH of Treatment 4 reduced significantly (p < 0.05) to 4.09 (±0.12) on day 10 (Table 4). However, it remained stable after 10 days of storage (Table 4).
Table 4. Mean pH (Mean ± SD) of probiotic yogurt treatments containing *Lactobacillus rhamnosus* GR-1 following 1, 10, 20, and 30 days of refrigerated storage.

| Treatment | 1 day  | 10 days | 20 days | 30 days | *p*-Value |
|-----------|--------|---------|---------|---------|-----------|
| 1         | 4.24 ± 0.00 | 4.21 ± 0.05 | 4.16 ± 0.04 | 4.15 ± 0.05 | 0.32      |
| 2         | 4.20 ± 0.00<sup>a</sup> | 4.10 ± 0.02<sup>b</sup> | 4.02 ± 0.02<sup>c</sup> | 3.97 ± 0.05<sup>d</sup> | 0.004     |
| 3         | 4.30 ± 0.14 | 4.06 ± 0.03 | 3.99 ± 0.03 | 3.95 ± 0.00 | 0.067     |
| 4         | 4.29 ± 0.14<sup>a</sup> | 4.09 ± 0.12<sup>b</sup> | 4.04 ± 0.04<sup>b</sup> | 3.97 ± 0.02<sup>b</sup> | 0.028     |

A one-way repeated-measures ANOVA and Fisher’s least significant difference test were used to analyse statistically significant differences (*p* < 0.05) between mean pH values of four probiotic yogurt treatments over 30 days of refrigerated storage. In a row, mean pH values for each treatment followed by the same superscript letter (a–d) are not significantly different (*p* > 0.05). (Treatment 1 indicates 100% cow milk (control); Treatment 2, 75% cow milk + 25% soymilk; Treatment 3, 50% cow milk + 50% soymilk; Treatment 4, 25% cow milk + 75% soymilk).

3.3. Sensory Analysis

3.3.1. Appearance

The mean hedonic scores for appearance from the sensory evaluation are depicted in Figure 1. The treatments containing a higher percentage of cow milk were found to be more appealing to participants. For instance, the mean score for Treatment 1, the control, was 7.33 (±1.31), which was significantly higher (*p* < 0.05) than all other treatments. A value of 7 on the hedonic scale corresponds with “like moderately”. The appearance of Treatment 2 was the next most liked amongst the treatments, with a mean hedonic score of 6.94 (±1.43), followed by Treatment 3, with a mean hedonic score of 6.37 (±1.68). A value of 6 on the hedonic scale corresponds with “like somewhat”. Treatment 4 scored the lowest for appearance, with a mean hedonic score of 5.83 (±1.80), which corresponds with “neither like nor dislike”. Treatments 2–4 were slightly off-white in appearance and were less viscous than the control, which may have not appealed to participants as a plain white colour (such as the colour of Treatment 1) is most commonly associated with yogurt.

![Appearance](image_url)

**Figure 1.** Panellists (*n* = 111) rated the appearance of four probiotic yogurt treatments using a nine-point hedonic scale ranging from 1 (dislike extremely) to 9 (like extremely). A one-way repeated-measures ANOVA and Fisher’s least significant difference test were used to analyse statistically significant differences (*p* < 0.05) between mean hedonic scores. Significant differences between treatments are denoted by different lowercase letters (a–d) above each bar graph. (Treatment 1 indicates 100% cow milk (control); Treatment 2, 75% cow milk + 25% soymilk; Treatment 3, 50% cow milk + 50% soymilk; Treatment 4, 25% cow milk + 75% soymilk).
3.3.2. Flavour

There were no significant differences (\( p > 0.05 \)) amongst the treatments in the panellists’ liking for flavour, and a mean score of 5 was achieved for all treatments, which corresponds with “neither like nor dislike” (Figure 2). Flavoured yogurt, typically with fruit pieces, is more commonly consumed by the North American population compared to plain yogurt, which is a staple in some parts of the Middle East and South Asia [26]. Some panellists indicated they were not in favour of the plain, sour taste and would prefer sweeter yogurt. These factors may help explain the neutral response of participants to the yogurt treatments in the study.

![Flavour](image)

**Figure 2.** Panellists (\( n = 111 \)) rated the flavour of four probiotic yogurt treatments using a nine-point hedonic scale ranging from 1 (dislike extremely) to 9 (like extremely). A one-way repeated-measures ANOVA and Fisher’s least significant difference test were used to analyse statistically significant differences (\( p < 0.05 \)) between mean hedonic scores. (Treatment 1 indicates 100% cow milk (control); Treatment 2, 75% cow milk + 25% soymilk; Treatment 3, 50% cow milk + 50% soymilk; Treatment 4, 25% cow milk + 75% soymilk).

3.3.3. Texture

Significant differences (\( p < 0.05 \)) were observed between treatments in the panellists’ preferences for the texture (Figure 3). Treatment 1 (control) received a mean score of 6.73 (±1.60) (“like somewhat”). This score was significantly higher (\( p < 0.05 \)) than the scores for Treatments 3 and 4, which were 5.80 (±2.00) and 6.16 (±1.94), respectively. The panellists’ liking for textures of Treatments 1 and 2 were comparable. However, the score for Treatment 2 (6.45 ± 1.66) was significantly higher (\( p < 0.05 \)) than that for Treatment 3 (5.80 ± 2.00), while Treatments 2 and 4 (6.16 ± 1.94) shared comparable scores for texture. Finally, the score for the texture of Treatment 3 (5.80 ± 2.00) was significantly lower (\( p < 0.05 \)) than that of 1 and 2, while it shared comparable scores with Treatment 4 (6.16 ± 1.94).

3.3.4. Overall Acceptability

There were no significant differences (\( p > 0.05 \)) amongst the treatments in the panellists’ rating for overall acceptability, and a score in the range of 5–6 was achieved for all treatments, which corresponds with “neither like nor dislike” to “like somewhat” (Figure 4). On page 2 of the questionnaire, participants could comment on their preference for each sample (data not shown). Almost 1 in 3 respondents indicated a liking for Treatment 4 and cited the mildly sweet and nutty flavour, lack of aftertaste, and thin texture as its appealing characteristics. Some panellists compared Treatment 4 with the other treatments and were in favour of the reduced sourness. One out of four panellists preferred Treatment 1, the control, over the other treatments due to the familiar white colour, sour and mildly
sweet flavour, and thick, yet smooth consistency. Treatment 3 was the least liked sample as it left an aftertaste and its texture was described as sticky and runny.

Figure 3. Panellists (n = 111) rated the texture of four probiotic yogurt treatments using a nine-point hedonic scale ranging from 1 (dislike extremely) to 9 (like extremely). A one-way repeated-measures ANOVA and Fisher’s least significant difference test were used to analyse statistically significant differences (p < 0.05) between mean hedonic scores. Significant differences (p < 0.05) between treatments are denoted by different lowercase letters (a-c) above each bar graph. (Treatment 1 indicates 100% cow milk (control); Treatment 2, 75% cow milk + 25% soymilk; Treatment 3, 50% cow milk + 50% soymilk; Treatment 4, 25% cow milk + 75% soymilk).

Figure 4. Panellists (n = 111) rated the overall acceptability of four probiotic yogurt treatments using a nine-point hedonic scale ranging from 1 (dislike extremely) to 9 (like extremely). A one-way repeated-measures ANOVA and Fisher’s least significant difference test were used to analyse statistically significant differences (p < 0.05) between mean hedonic scores. (Treatment 1 indicates 100% cow milk (control); Treatment 2, 75% cow milk + 25% soymilk; Treatment 3, 50% cow milk + 50% soymilk; Treatment 4, 25% cow milk + 75% soymilk).

4. Discussion

The primary aim of the present study was to propose a new layout for a fermented, soy and cow milk-based functional food and to determine the potential of this formulation to serve as a suitable substrate for the growth and proliferation of L. rhamnosus GR-1. This option is postulated to provide
an alternative medium for probiotic delivery, thereby making the functional food market accessible to a wider segment of the population, such as to individuals who are seeking lower cholesterol, saturated fat, and lactose alternatives to purely cow milk-based probiotic yogurt, as well as in lower income countries where cow milk is costly and legumes are more readily accessible [2].

Despite the growing interest in plant-based foods such as soy, the sensory appeal of soymilk is low amongst most consumers due to its beany flavour, which is attributed to the presence of unsaturated fatty acids and lipoxygenases that give rise to volatile compounds (hexanal and pentanal) [2,27,28]. Additionally, some individuals report abdominal discomfort, diarrhoea, and flatulence following soybean consumption due to its oligosaccharides (raffinose and stachyose), which are indigestible in the human intestinal tract [11,21,28]. Other organoleptic properties, such as a brownish colour, chalky texture, and thin mouthfeel, also promote limited acceptance of soy-based foods [11]. Thus, our secondary objective was to conduct a sensory evaluation to elucidate whether this new soy and cow-milk based formulation offers considerable appeal to consumers.

During 6 h of fermentation, all treatments containing soymilk (i.e., Treatments 2–4) supported the growth of *L. rhamnosus* GR-1 and allowed viable counts of at least 10⁸ CFU/mL to be achieved and maintained over 30 days of cold storage at 4 °C. This value surpassed the minimum recommended microbial load of 10⁶ CFU/mL for probiotic effect [16]. The results of Treatments 2–4 were comparable to that of the control (Treatment 1), thereby exhibiting the incorporation of soymilk and durations of fermentation and storage did not induce a loss in bacterial viability. Previous studies also demonstrated successful inoculation of *L. rhamnosus* GR-1 in non-dairy matrices, such as fruit and vegetable juices and fermented rice pudding to determine their suitability as carriers of probiotics [24,25,29,30]. Mean viable microbial loads of at least 10⁷–10⁹ CFU/mL were reached in all cases [24,25,29,30].

An investigation by Rostami et al. yielded similar results, wherein cow milk-based yogurt formulations supplemented with either 20%, 40%, or 60% soymilk allowed for the microbial counts of *L. acidophilus*, *L. casei*, and yogurt starter culture (i.e., *L. delbrueckii ssp. bulgaricus* and *Streptococcus thermophilus*) to be comparable to the counts of these strains in 100% cow milk-based yogurt [31]. When analysing the growth of either *L. rhamnosus* GG or *L. johnsonii* La-1 in conjunction with yogurt starter culture in soy yogurt, Farnworth et al. found the microbial load of these probiotic bacteria to be 5 and 3 times better in the soy yogurt compared to cow milk yogurt, respectively, and *L. rhamnosus* GG exhibited a better growth potential in the soy yogurt compared to *L. johnsonii* La-1 [4]. Likewise, Liu et al. introduced a probiotic soy cheese fortified with *L. rhamnosus* 6013 [32]. The microbial count of the probiotic strain surpassed the minimum level of 10⁶ CFU/mL for therapeutic effect, reaching 10⁶–10⁹ CFU/mL after 6 h of fermentation, and an increase in acidity of the product was recorded after 30 days of cold storage at 10 °C [32]. The levels of stachyose and raffinose were also significantly reduced (*p < 0.05*) due to metabolism by the probiotic bacteria [32].

Previous studies reported an improvement in nutritional value through fortification of dairy-based milk with soymilk [33,34]. Nurliyani et al. developed kefir using varying combinations of soy and goat milk and found the microbial counts of lactic acid bacteria to be comparable between the mixture and goat milk alone [33]. Additionally, the 50–50 combination of soy and goat milk contained higher levels of an omega-9 fatty acid, oleic acid, compared to 100% goat milk, and lower concentrations of certain saturated fatty acids, specifically caproic, heptadecanoic, and behenic acids [33]. Ghoneem et al., who developed bioyogurt using various blends of buffalo, cow, and soymilk with ABT-5 culture (consisting of *Streptococcus thermophilus*, *L. acidophilus*, and *Bifidobacterium* BB-12), also reported significant improvements (*p < 0.05*) in linoleic and α-linolenic acid concentrations and a decrease in the level of saturated fatty acids upon replacement of 25% buffalo or cow milk with soymilk [34].

Upon evaluation of the pH profile of the treatments in the present study during fermentation and cold storage, formulations containing soymilk (i.e., Treatment 2–4) experienced a significant increase (*p < 0.05*) in acidity. While the decline in pH was steeper in the soy-based formulations compared to Treatment 1, the final pH values of all treatments were similar at each point during fermentation and storage. This observation is similar to that noted by Farnworth et al., who compared the growth of the
yogurt starter culture in soy yogurt with its growth in cow milk yogurt [4]. The steeper rise in acidity of the soy yogurt was attributed to the lower buffering capacity of soy compared to cow milk due to its different protein composition and the physicochemical properties of these proteins [4,6]. Champagne et al.’s results were also consistent with the present study as they reported a greater acidification rate in soy yogurt inoculated with *L. rhamnosus* R0011 compared to cow milk yogurt [35].

However, Ghoneem et al. reported different observations, wherein a biyo yogurt formulation containing soymilk alone had a lower titratable acidity compared to buffalo and cow milk, and a mixture of soymilk with either beverage had an even lower titratable acidity level [34]. Similarly, Osman and Razig evaluated the quality attributes of four soymilk and cow milk formulations fermented using yogurt starter culture [32]. Interestingly, the 25% soymilk and 75% cow milk blend produced the lowest pH value of 3.3 during fermentation, followed by the 50–50 mixture, and finally the 75% soymilk and 25% cow milk blend [36].

Consistent with our results during cold storage, Osman and Razig also noted an increase in acidity during the 20-day storage period [36]. A similar observation of a drop in pH during cold storage was described by Mondragón-Bernal et al. in synbiotic soy yogurt formulation consisting of *L. rhamnosus* LR32 and standard probiotic inoculum [37]. pH values in the range of 5.5 to 6.0 are conducive to the proliferation of strains belonging to the *Lactobacillus* genus; however, they can sustain pH values as low as 3.7–4.3 [16]. Thus, while a steep decline in pH (i.e., lower buffering capacity) may be concerning due to the potential adverse effects on microbial counts, the observation in the present study was not detrimental as bacterial viability was maintained during fermentation and storage [15,20].

Through the sensory evaluation, it was determined that panellists had a greater preference for the texture and appearance of Treatment 1, while scores for the flavour and overall acceptability of the treatments were comparable and in the “neither like nor dislike” to “like slightly” range of the hedonic scale. Similarly, Osman and Razig reported a 75% cow milk and 25% soymilk blend attained the best scores for appearance, flavour, texture, and overall acceptability compared to treatments with a higher proportion of soymilk [36]. Organoleptic properties are the main setback for the adoption of soy-based products and, typically, consumers prefer cow milk yogurt over soy yogurt due to its colour and texture [28]. Incorporation of natural and synthetic flavouring agents, fruit pulps, fructooligosaccharides, and essences may help attenuate the undesirable taste, while enhancing the nutrient profile of the yogurt [6,38,39]. Additionally, response surface technology, as demonstrated by Kumar and Mishra through the development of mango soy fortified yogurt, may also help mask the beany taste of soymilk [40].

While soy and cow milk share similar nutrient profiles compared to other plant-based milk alternatives, using a combination of soy and cow milk to produce a functional food integrates the favourable characteristics of both foods [2,3,39]. For instance, the final product supplies an extra boost in protein quality, including sulphur-containing amino acids, methionine, cysteine, and cystin, which are present in limited quantities in soymilk [2,39]. Further, the isoalloxazine, B vitamin, soluble fibre, magnesium, riboflavin, niacin, and calcium content of the mixture is improved [2,3,39–42]. The fat and protein content of cow milk, as well as its buffering capacity, can be leveraged to fuel bacterial metabolism, survival, and proliferation [20]. Additionally, the less desirable features of the individual components are minimised, such as the poor palatability of soymilk and high lactose, caloric, carbohydrate, saturated fat, and cholesterol content of cow milk [41]. In combination, these factors allow for a nutritionally similar alternative to both cow milk and soymilk to be produced with distinct nutritional advantages, allowing consumers to derive a range of unique health benefits [40,42,43].

Future research should explore the retention of the functional efficacy of *L. rhamnosus* GR-1 in this new matrix through simulated gastric and intestinal juice models, and whether the viability observed during fermentation and storage is adequately preserved in the gastrointestinal tract for the therapeutic effects of *L. rhamnosus* GR-1 to be acquired [43]. Additionally, the incorporation of prebiotics, such as inulin, should be investigated for their added support to *L. rhamnosus* GR-1 during transit through the gastrointestinal tract, as well as the effect of inulin on the viscosity of soy yogurt [44]. Finally,
the potential role of the oligosaccharides, which are found in soymilk, as prebiotics for \textit{L. rhamnosus} GR-1 warrants investigation [44].

5. Conclusions

Probiotic yogurt produced through fortification of cow milk with soymilk exhibited acceptable carrier properties when inoculated with \textit{L. rhamnosus} GR-1, which attained and maintained mean viable counts of \(10^8\) CFU/mL in all soy-based treatments, thereby exceeding the minimum requirement of \(10^6\) CFU/mL for therapeutic effects. This new layout for \textit{L. rhamnosus} GR-1, which represents a merger between plant and dairy-based matrices, provides a wider and more accessible range of probiotic products as a means of relief from urogenital infections and various forms of gastrointestinal distress for patients. It may particularly be beneficial for individuals in lower income countries and for those who are seeking lower cholesterol, saturated fat, and lactose alternatives to dairy-based matrices, without compromising on the sensory appeal of the functional food.

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