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Lactobacillus rhamnosus GR-1 in Fermented Rice Pudding Supplemented with Short Chain Inulin, Long Chain Inulin, and Oat as a Novel Functional Food

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Abstract: *Lactobacillus rhamnosus* GR-1 is a probiotic that has been shown to reduce the risk of urogenital problems and urinary tract infections. Rice pudding is a popular gluten-free dairy product, and could be a vehicle to deliver *L. rhamnosus* GR-1 to a broader population. The purpose of this study was to investigate the growth and viability of *L. rhamnosus* GR-1 in six fermented rice pudding samples, each one supplemented with one type of prebiotic (short-chain inulin-2% *w*/*w*, 4% *w*/*w*; long-chain inulin-2% *w*/*w*, 4% *w*/*w*) and oat-0.5% *w*/*w*, 1% *w*/*w*, along with control, over a 21-day storage period. The objective was to determine if the supplementation would have a positive effect on the microbial viability of *L. rhamnosus* GR-1, and to evaluate the sensory properties of the samples. All of the samples had viable levels of *L. rhamnosus* GR-1. Bacterial counts were at least 1×10^8 CFU/mL over the 21-day storage period. The probiotic rice pudding sample supplemented with 4% *w*/*w* short-chain inulin had the highest hedonic score for flavour, sweetness, texture, and overall acceptability. This study shows that the addition of short-chain inulin, long-chain inulin, and oat had no adverse supplementation effects on the viability of L. *Rhamnosus* GR-1. There is the potential for the production of a novel functional food.

Keywords: *Lactobacillus rhamnosus* GR-1; probiotic rice pudding; prebiotic; short-chain inulin; long-chain inulin; oat

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1. Introduction

Probiotics are live non-pathogenic bacteria which benefit health when ingested [1]. Therapeutic benefits can vary according to specific species and strain. The most frequently used types of probiotics are *Lactobacillus* and *Bifidobacterium* species [2]. *Lactobacillus rhamnosus* GR-1 is bile-resistant and survives the passage through the gastrointestinal tract [3,4]. It has been found to relieve urogenital problems and urinary tract infections by colonizing intestinal and urogenital cells [5–7]. Most probiotics are available as over-the-counter items, and as fermented dairy products, such as yogurt and kefir [1,2]. In recent years, there has been an increase in the consumer awareness of and demand for probiotic dairy products [2,8,9]. Probiotic products need to contain at least 10^6 colony-forming units (CFU) per gram for the duration of the product's shelf life in order to transfer beneficial effects to the host [10]. Acid production by probiotic culture, oxygen permeability through the package, and physical condition of product storage can decrease the viability and sensory characteristics of a probiotic product before the end of its shelf life [11,12]. In this study, *L. rhamnosus* GR-1 was the technological starter culture used to ferment the rice pudding samples. With the addition of a technological starter culture to rice pudding samples and incubation of rice pudding samples for 5 h, fermented rice pudding samples were created with a slight sour taste. All fermented rice pudding samples had a decrease in the pH over the storage period of 21 days.

Rice pudding is a popular gluten-free dairy product that is enjoyed throughout the world [13]. At present, there are no probiotic fermented rice puddings on the market that are known to the authors. Additionally, there is little information and research regarding rice pudding that is supplemented with probiotics and fermented. Few research studies exist looking into ways of creating probiotic cereal-based products [14–16]. There is a potential for the dairy industry to provide a novel food product that incorporates *L. rhamnosus* GR-1 probiotic into a rice pudding, making the rice pudding fermented and thus more beneficial to overall health, especially to people with urogenital problems and urinary tract infections. Urogenital infections affect approximately 1 billion people per year [6].

Prebiotics are non-digestible carbohydrates which tend to induce the growth and viability of gastrointestinal bacteria [17,18]. Some studies found a positive effect of prebiotics on probiotic growth and vitality in dairy and non-dairy products [14,16,19,20]. Most prebiotics are extracted from plant materials, and have the benefit of being a soluble dietary fibre [17]. Some of the most popular prebiotics used in the food industry are short-chain inulin, long-chain inulin, β-glucan in oat, and pectin [21]. There is research suggesting that prebiotics can lower the incidence of colon cancer, improve calcium absorption, reduce total and low-density lipoprotein (LDL)cholesterol, and increase regularity [18,21,22].

Inulin is a prebiotic and component of fruits and vegetables. The most frequently used plant for producing inulin is chicory root [20]. Inulin is a highly fermentable dietary fibre that increases the activity of *L. rhamnosus* [20]. In the food industry, inulin is used for its technological properties as a low-calorie sweetener, fat substitute, and texture modifier [20]. The properties of inulin are linked to its degree of polymerization (DP), where long-chain inulin has 23–25 units of DP, and short-chain inulin has fewer than 11 units of DP. Long-chain inulin is more thermally stable, less soluble, and more viscous than short-chain inulin [20,23]. Different DPs in inulin can have different effects on probiotic dairy products [20,23]. For example, a study of short-chain and long-chain inulin in a probiotic yogurt revealed that short-chain inulin received higher scores for odour, taste, and overall acceptability in a sensory evaluation than the yogurt with long-chain inulin [20]. The viability of bacteria in yogurt with short-chain inulin was also enhanced [20].

Oat is a major source of β-glucan, which acts as a prebiotic [24]. The soluble fibre in oat has been shown to control diabetes, lower cholesterol and risk of certain cancers, and relieve constipation [24,25]. Some studies found that probiotic bacterial growth was enhanced with the addition of oats [24,26].

Research examining the growth and viability of *L. rhamnosus* GR-1 in fermented rice pudding that is fortified with prebiotics will be important, as there are no studies that have incorporated *L. rhamnosus* GR-1 in rice pudding. The formulation of novel functional foods is very interesting in the field of food science and technology, and this study is intended to develop and evaluate a fermented dairy product for the delivery of a probiotic with viable bacterial counts and acceptable sensory characteristics. The objectives of this study were to determine the effect of short-chain inulin, long-chain inulin, and oat at different concentrations on the growth and viability of *L. rhamnosus* GR-1 in fermented rice pudding over a 21-day storage period, and to evaluate the sensory properties of these new dairy products.

2. Materials and Methods

2.1. Probiotic Stock Solution Preparation

L. rhamnosus GR-1 (10% *w*/*v*) (Urex Biotech Inc., London, ON, Canada) was inoculated into sterilized de Man, Rogosa, and Sharpe (MRS) broth (EMD Chemicals Inc., Gibbstown, NJ, USA) and was anaerobically incubated using a gas pack system (BD GasPak™ EZ Anaerobe Container System, Becton Dickinson & Co., Sparks, BD, USA) at 38 °C for 24 h. Probiotic stocks were prepared on a routine basis in MRS broth and stored in a 4 °C refrigerator.

2.2. Probiotic Starter Culture Preparation

Skim milk (0.1% milk fat) (Neilson® Trutaste® Microfiltered Skim Milk, Saputo Inc., Montreal, QC, Canada) was autoclaved at 121 °C at 15 PSI for 15 min. When milk was cooled down to 37 °C, 1% *w*/*v* of probiotic stock solution of *L. rhamnosus* GR-1 was added and mixed well, and then anaerobically incubated using the gas pack system (BD GasPak™ EZ Anaerobe Container System, Becton Dickinson & Co., Sparks, BD, USA) at 37 °C for 24 h. The probiotic sample was then placed in a 4 °C refrigerator overnight.

2.3. Rice Pudding Preparation

2.3.1. Rice Pudding Control

Skim milk (0.1% milk fat) (Neilson® Trutaste® Microfiltered Skim Milk, Saputo Inc., Montreal, QC, Canada) was heated along with medium grain white rice (120 g per 1 L of milk) (Calrose Rice, No Name®, No Frills chain grocery store, London, ON, Canada) and sucrose (20 g per 1 L of milk). It was heated to 95–98 °C and kept at that temperature for 30 min. The sample was cooled down to 37 °C before 4% probiotic starter culture was added. The rice pudding sample was well-mixed, covered, and incubated at 37 °C for 5 h. The fermented sample was kept at 4 °C for 21 days of storage.

2.3.2. Rice Pudding Fortified with Short-Chain Inulin

Two samples of rice pudding fortified with short-chain inulin were made, 2% *w*/*w* short-chain inulin and 4% *w*/*w* short-chain inulin. Skim milk (0.1% milk fat) (Neilson® Trutaste® Microfiltered Skim Milk, Saputo Inc., Montreal, QC, Canada) was heated along with medium grain white rice (120 g per 1 L of milk) (Calrose Rice, No Name®, No Frills chain grocery store, Canada), sucrose (20 g per 1 L of milk) and short-chain inulin (Orafti® P95 Oligofructose Powder, Quadra Chemicals, Burlington, ON, Canada). The samples were heated to 95–98 °C and kept at that temperature for 30 min. They were cooled down to 37 °C before 4% probiotic starter culture was added to each sample. The rice pudding samples were well-mixed, covered, and incubated at 37 °C for 5 h. The fermented samples were kept at 4 °C for 21-days of storage.

2.3.3. Rice Pudding Fortified with Long-Chain Inulin

Two samples of rice pudding fortified with long-chain inulin were made, 2% *w*/*w* long-chain inulin and 4% *w*/*w* long-chain inulin. Skim milk (0.1% milk fat) (Neilson® Trutaste® Microfiltered Skim Milk, Saputo Inc., Montreal, QC, Canada) was heated along with medium grain white rice (120 g per 1 L of milk) (Calrose Rice, No Name®, No Frills chain grocery store, Canada), sucrose (20 g per 1 L of milk) and long-chain inulin (Orafti® HP Inulin Powder, Quadra Chemicals, Burlington, ON, Canada). The samples were heated to 95–98 °C and kept at that temperature for 30 min. They were cooled down to 37 °C before 4% probiotic starter culture was added to each sample. The rice pudding samples were well mixed, covered, and incubated at 37 °C for 5 h. The fermented samples were kept at 4 °C for 21 days of storage.

2.3.4. Rice Pudding Fortified with Oat

Two samples of rice pudding fortified with oat were made: 0.5% *w*/*w* oat and 1% *w*/*w* oat. Skim milk (0.1% milk fat) (Neilson® Trutaste® Microfiltered Skim Milk, Saputo Inc., Montreal, QC, Canada) was heated along with medium grain white rice (120 g per 1 L of milk) (Calrose Rice, No Name®, No Frills chain grocery store, Canada), sucrose (20 g per 1 L of milk), and oat (One-minute 100% Whole Grain Oats, No Name®, No Frills chain grocery store, Canada). The samples were heated to 95–98 °C and kept at that temperature for 30 min. They were cooled down to 37 °C before 4% probiotic starter culture was added to each sample. The rice pudding samples were well-mixed, covered, and incubated at 37 °C for 5 h. The fermented samples were kept at 4 °C for 21 days of storage.

2.4. Microbial Analysis

Enumeration of *L. rhamnosus* GR-1 from all seven fermented rice pudding samples was conducted on days 1, 7, 14, and 21 of the refrigerated storage at 4 °C using serial dilution and subsequent plating. There were two replications for each of the seven fermented rice pudding samples. All samples were diluted in sterile 0.85% saline solutions of 10^{-1}, 10^{-3}, 10^{-5}, 10^{-6}, 10^{-7} dilution factors. For each fermented rice pudding sample, 0.1 mL of the 10^{-6} and 10^{-7} dilutions were enumerated on two separate MRS agar plates and anaerobically incubated (BD GasPak™ EZ Anaerobe Container System, Becton Dickinson & Co., Sparks, BD, USA) at 37 °C for 48 h. MRS agar plates were prepared containing 5.22% MRS broth (EMD Chemicals Inc., Gibbstown, NJ, USA), 1.5% agar (EMD Laboratories, 2695 North Sheridan Way, Suite 200, Mississauga, ON, Canada), and 0.015 g/L fusidic acid (Enzo Life Sciences, 10 Executive Blvd, Farmingdale, NY, USA). After incubation, viable microbial counts on MRS agar plates were determined and recorded as colony-forming units (CFU) per mL.

2.5. Analysis of pH

The pH of all rice pudding samples was measured on days 1, 7, 14, and 21 at 4 °C using a VWR® SympHony™ B10P Benchtop pH Meter (VWR International, Radnor, PA, USA).

2.6. Sensory Evaluation

Four fermented probiotic rice pudding samples were prepared (rice pudding fortified with 4% *w*/*w* short-chain inulin, rice pudding fortified with 4% *w*/*w* long-chain inulin, rice pudding fortified with 0.5% *w*/*w* oat, and the control rice pudding). Sensory evaluation was reviewed and approved by the Research Ethics Board of Western University, London, ON, Canada. There were a total of 104 untrained taste panellists between the ages of 18 and 55, who gave written informed consent to participate in the study. Each panellist received a tray of four coded fermented probiotic rice pudding samples in a random order in the individual booths (Sensory Lab, Brescia University College at Western University, London, ON, Canada). The sample sizes were large enough so that panellists could retaste the products, as they desired. Panellists were instructed to cleanse their palate with water between the samples in order to reduce the overlap of flavours. Using a nine-point hedonic scale [27], panellists rated each sample based on the characteristics of appearance, flavour, sweetness, texture, and overall acceptability. The hedonic scale had nine categories, and each point on the hedonic scale was assigned a value ranging from 9 "like extremely" to 1 "dislike extremely". Panellists also filled a questionnaire regarding: willingness of purchase of these fermented probiotic rice puddings, frequency of rice pudding consumption, preferred rice pudding consistency, and willingness to purchase fermented probiotic rice pudding.

2.7. Statistical Analysis

Statistical analyses were conducted using SAS® 9.4 Software (SAS Institute Inc., Cary, NC, USA). A one-way repeated measure analysis of variance (ANOVA) was used to analyse the relationship between sample and the effect of shelf life on viable probiotic bacteria over time. A repeated measures ANOVA and Tukey's test ($p < 0.05$) were used to test for significant differences between mean scores. A *p*-value of <0.05 was considered to be statistically significant. Statistical analyses for the sensory evaluation of probiotic rice pudding samples were also conducted using SAS® 9.4 Software (SAS Institute Inc., Cary, NC, USA). Among-sample comparisons were made using ANOVA. Tukey's studentized range test was used to adjust for multiple comparisons when examining pairwise differences.

3. Results

3.1. Microbial Counts and Stability

All of the fermented rice pudding samples had viable levels of *L. rhamnosus* GR-1, where bacterial counts were at least 1×10^8 CFU/mL over the 21-day storage period. The addition of long-chain inulin, short-chain inulin, and oat did not decrease the viability and survival of the bacteria when compared to the control sample (Table 1).

Table 1. Viable counts (Mean ± SD) of *L. rhamnosus* GR-1 in probiotic rice pudding supplemented with 2% short-chain inulin (2% SCI), 4% short-chain inulin (4% SCI), 2% long-chain inulin (2% LCI), 4% long-chain inulin (4% LCI), 0.5% oat (0.5% O), and 1% oat (1% O) compared to control (CON) sample after 1, 7, 14, and 21 days of storage.

	Mean Counts ($\times 10^8$ CFU/mL) of *L. rhamnosus* GR-1							
Storage	**CON**	**2% SCI**	**4% SCI**	**2% LCI**	**4% LCI**	**0.5% O**	**1% O**	***p*-Value**
Day 1	19.6 ± 1.3	20.4 ± 4.5	25.6 ± 9.7	24.1 ± 2.2	21.1 ± 12.0	67.7 ± 41.3	30.8 ± 5.9	0.157
Day 7	44.0 ± 8.2	40.2 ± 0.9	43.4 ± 6.8	65.8 ± 32.2	52.5 ± 14.2	59.5 ± 20.9	66.4 ± 8.5	0.500
Day 14	27.7 ± 6.2	40.1 ± 9.1	47.0 ± 3.0	46.1 ± 20.4	40.1 ± 14.4	46.0 ± 13.4	47.7 ± 13.5	0.594
Day 21	26.9 ± 0.8	36.4 ± 5.1	43.1 ± 13.1	42.6 ± 0.4	39.8 ± 13.2	40.4 ± 14.6	35.6 ± 0.3	0.526

CFU-colony forming units. A repeated-measures ANOVA and Tukey's test ($p < 0.05$) were used to test for significant differences between mean scores. A p-value of < 0.05 was considered to be statistically significant.

The initial mean counts ($\times 10^8$ CFU/mL) of *L. rhamnosus* GR-1 at day 1 for rice pudding control (CON), rice pudding with 2% short-chain inulin (2% SCI), rice pudding with 4% short-chain inulin (4% SCI), rice pudding with 2% long-chain inulin (2% LCI), rice pudding with 4% long-chain inulin (4% LCI), rice pudding with 0.5% oat (0.5% O), and rice pudding with 1% oat (1% O) were 19.6 ± 1.3, 20.4 ± 4.5, 25.6 ± 9.7, 24.1 ± 2.2, 21.1 ± 12.0, 67.7 ± 41.3, and 30.8 ± 5.9, respectively. Among fermented probiotic rice pudding samples, differences were not significant on any of the four days (Table 1). The results also showed no adverse effects of short-chain inulin, long-chain inulin, or oat on the viability of *L. rhamnosus* GR-1. Although results were not statistically significant ($p > 0.05$), they showed that CON, 2% SCI, 2% LCI, 4% LCI, and 1% O rice pudding samples had significantly increased mean microbial counts of *L. rhamnosus* GR-1 by day 7 (Table 1). Mean microbial counts on day 7 were: 44.0 ± 8.2, 40.2 ± 0.9, 65.8 ± 32.2, 52.5 ± 14.2, and 66.4 ± 8.5, respectively.

Pairwise comparisons of storage days using repeated-measures ANOVA (p-values) for each probiotic rice pudding separately indicated no significant differences in storage days 1, 7, 14, and 21, for probiotic rice pudding fortified with 2% SCI, 4% SCI, 2% LCI, 0.5% O and 1% O. However, there were significant differences in the bacterial counts with probiotic rice pudding control for day 1 vs. day 7 ($p = 0.019$), where the microbial count had almost doubled. Another significant difference in the bacterial counts was seen with probiotic rice pudding fortified with 4% LCI for day 1 vs. day 7 ($p = 0.020$), day 1 vs. day 14 ($p = 0.050$), and day 1 vs. day 21 ($p = 0.050$). It peaked at day 7, and then had a decrease of microbial counts, but was still higher than at day 1. The results also show that all of the probiotic rice pudding samples had viable mean microbial counts of *L. rhamnosus* GR-1 until the end of the rice pudding shelf life, at day 21. Mean microbial counts at day 21 were all above 1×10^8 CFU/mL.

3.2. pH in Fermented Probiotic Rice Pudding Samples

Bacterial counts and pH were measured from the same cup on days 1, 7, 14, and 21. The analyses were performed using SAS 9.4 (SAS Institute Inc., Cary, NC, USA). A p-value < 0.05 was considered to be statistically significant. The results showed that all fermented probiotic rice puddings had a decrease in the pH over the storage period of 21 days (Figure 1). Among fermented probiotic rice pudding, differences were not statistically significant on days 1, 14, or 21 ($p > 0.05$). For day 7, the overall comparison was statistically significant ($p = 0.034$).

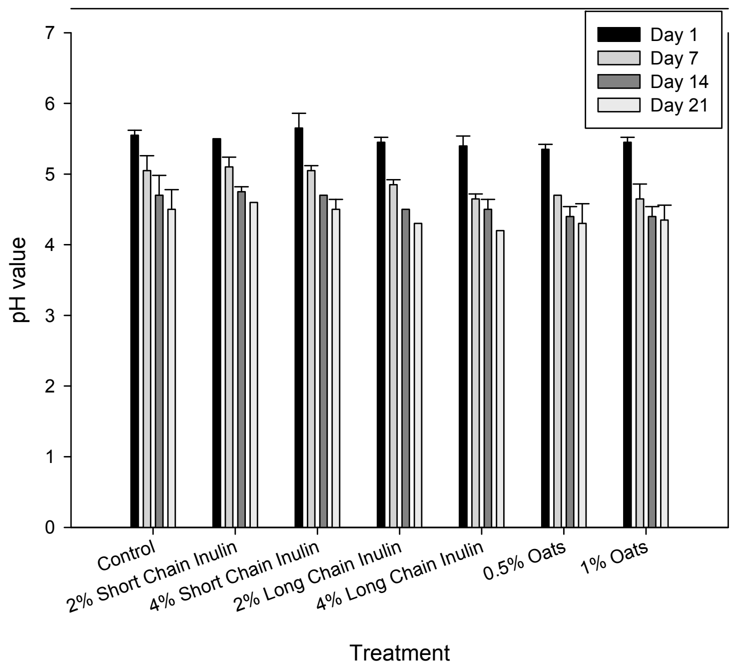

Figure 1. pH-comparisons of configurations within days, for the fermented probiotic rice pudding control, fermented probiotic rice pudding fortified with 2% short-chain inulin, fermented probiotic rice pudding fortified with 4% short-chain inulin, fermented probiotic rice pudding fortified with 2% long-chain inulin, fermented probiotic rice pudding fortified with 4% long-chain inulin, fermented probiotic rice pudding fortified with 0.5% oat, and fermented probiotic rice pudding fortified with 1% oat. pH measurements were taken on day 1, 7, 14, and 21.

3.3. Sensory Evaluation

The in-house taste panel determined the type and percentage of probiotic rice puddings used for sensory evaluation. In an attempt to not overwhelm the panellists, it was decided that four probiotic rice pudding samples were to be used for the sensory evaluation. The four probiotic rice pudding samples were chosen based on their most distinct sensory properties. The panel chose the probiotic rice puddings supplemented with: 4% SCI, 4% LCI, and 0.5% O, along with CON. The differences between the mean appearance ratings of four different probiotic rice puddings are shown in Figure 2.

The mean appearance score for CON was 6.67, which was slightly higher than other samples. The hedonic score of 6.0 corresponds to "like slightly" of the samples. There was no significant difference between mean appearance scores of CON, 4% LCI, and 0.5% O. The least liked appearance score of 5.51 was with 4% SCI. It could be due to the colour, as the 4% SCI had a slight yellow shade which may have not been viewed favourably by panellists.

The mean flavour scores of CON, 4% SCI, 4% LCI, and 0.5% O are shown in Figure 3. The mean flavour score of 4% SCI received a significantly higher score ($p < 0.05$) than the other samples (Figure 3).

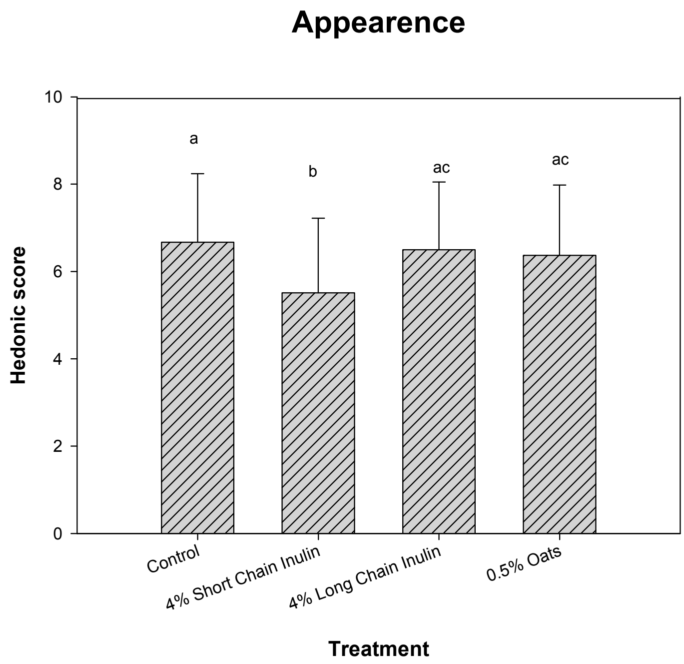

Figure 2. Mean appearance scores (±SD) on a hedonic scale from all judges of all four probiotic rice puddings. Means followed by the same letter are not significantly different according to Tukey's studentized range test ($p < 0.05$).

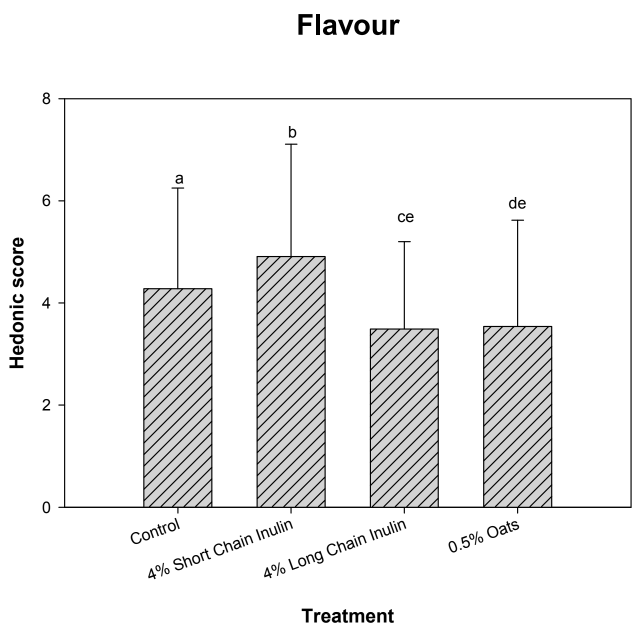

Figure 3. Mean flavour scores (±SD) on a hedonic scale from all judges of all four probiotic rice puddings. Means followed by the same letter are not significantly different according to Tukey's studentized range test ($p < 0.05$).

The higher mean flavour score of the 4% SCI sample could be due to the natural sweetness of the short-chain inulin. These results are similar to the findings of Canbulat et al. [20], whose study of short-chain and long-chain inulin in a probiotic yogurt revealed that short-chain inulin received higher scores for odour, taste, and overall acceptance than the yogurt with long-chain inulin. This trend is similar to the mean sweetness scores (Figure 4) and mean texture scores (Figure 5), with 4% SCI receiving a significantly higher score ($p < 0.05$) than other samples.

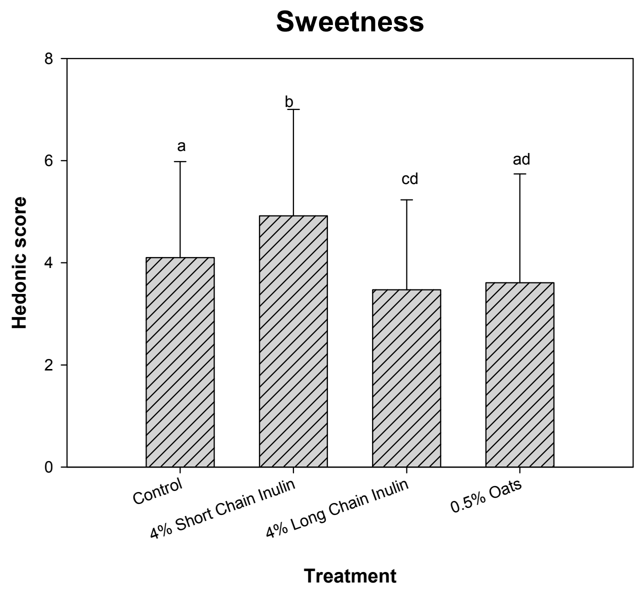

Figure 4. Mean sweetness scores (±SD) on a hedonic scale from all judges of all four fermented probiotic rice puddings. Means followed by the same letter are not significantly different according to Tukey's studentized range test ($p < 0.05$).

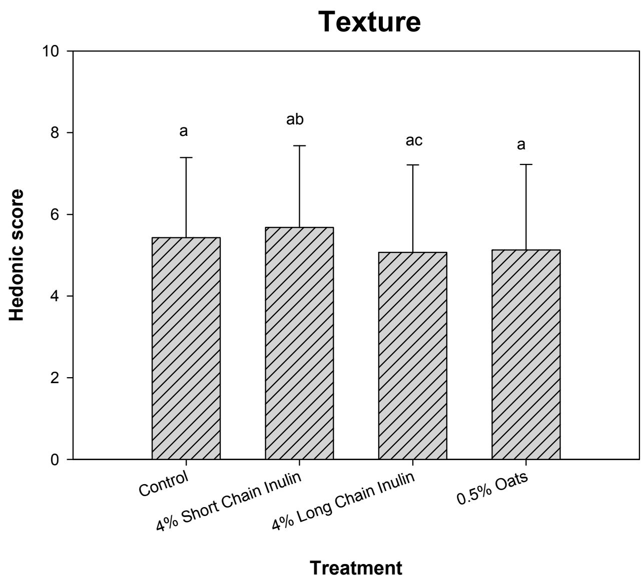

Figure 5. Mean texture scores (±SD) on a hedonic scale from all judges of all four fermented probiotic rice puddings. Means followed by the same letter are not significantly different according to Tukey's studentized range test ($p < 0.05$).

The mean overall acceptability scores of CON, 4% SCI, 4% LCI, and 0.05% O are shown in Figure 6. It shows that CON and 4% SCI are statistically significantly different ($p < 0.05$) from 4% LCI and 0.05% O. The mean acceptability score of 4% SCI received a slightly higher score of 5.09 than that of CON with 4.66.

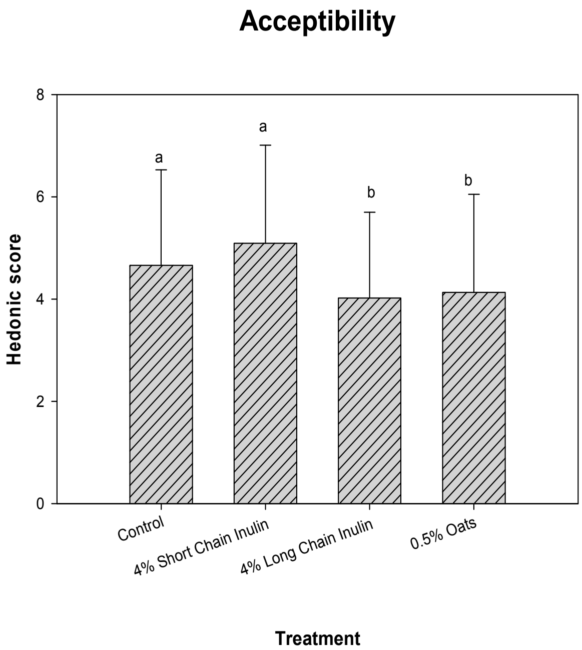

Figure 6. Mean acceptability scores (±SD) on a hedonic scale from all judges of all four fermented probiotic rice puddings. Means followed by the same letter are not significantly different according to Tukey's studentized range test ($p < 0.05$).

Through sensory evaluation questionnaire analysis (data not shown), 36% of panellists had never previously consumed rice pudding prior to this sensory evaluation. When panellists were asked whether they would purchase any of the four fermented samples that were offered, 60% indicated they would. Out of these 60% of panellists, 46% chose the 4% SCI sample, 24% chose the CON sample, 24% chose the 0.5% O sample, and 6% chose the 4% LCI sample. The majority of panellists that indicated that they would not purchase any of these four fermented probiotic rice puddings reasoned that the rice puddings were not sweet enough.

4. Discussion

The objective was to determine the effect of short-chain inulin, long-chain inulin, and oat supplementation on the growth and viability of *L. rhamnosus* GR-1 in fermented rice pudding over a 21-day storage period, and to evaluate the sensory properties of these new dairy products. All of the rice pudding samples had viable levels of *L. rhamnosus* GR-1, where bacterial counts were at least 1×10^8 CFU/mL over 21-day storage period. Among probiotic rice pudding samples, differences were not significant on any of the four days (Table 1). The results also showed no adverse supplementation effects on viability of the *L. rhamnosus* GR-1. Although results were not statistically significant ($p > 0.05$), they showed that CON, 2% SCI, 2% LCI, 4% LCI, and 1% O rice pudding samples had significantly increased mean microbial counts of *L. rhamnosus* GR-1 by day 7 (Table 1).

A few studies have reported positive outcomes in using prebiotics with probiotic culture [16,20,24,28–32]. The prebiotics seem to enhance the growth and viability of probiotics. A study done by Helland et al. [14] found that *L. rhamnosus GG* had the highest microbial cell count in fermented milk-based and water-based puddings that contained 75% rice flour and 25% maize flour.

In our study, the addition of oat did not increase bacterial counts in fermented rice pudding samples as predicted, based on the above studies. The *p*-value was not statistically significant, but the addition of oat showed no adverse effects on the viability of L. *rhamnosus* GR-1. The addition of oat did not affect the appearance or texture of fermented rice pudding samples. Since oat is not a naturally sweet prebiotic, it scored lower on the hedonic scale when compared to short-chain inulin.

The effect of inulin on the viability of probiotic bacteria in dairy products is positive, and seems to increase the microbial cell count. Our research shows that the addition of inulin as a prebiotic showed no adverse effects on the viability of bacteria in all rice pudding samples, for the entire storage period. Other studies of inulin and its effect on probiotic bacteria in dairy products shows that the addition of inulin contributes to viable cell counts of bacteria [20,30–32].

In our study, the fermented probiotic rice pudding sample fortified with 4% w/w short-chain inulin had the highest hedonic score for flavour, sweetness, texture, and overall acceptability. Other sensory evaluation studies regarding supplementation of inulin in probiotic dairy products showed similar results [20,33]. A study done by Canbulat et al. [20] reports on the higher score for odour, taste, and overall acceptability for a yogurt with short-chain inulin than for a yogurt with long-chain inulin. A study done by Aryana et al. shows that flavour scores for yogurt with short-chain inulin were significantly higher than for yogurt with long-chain inulin [33]. This could be due to short-chain inulin having a third to half the sweetness of sugar [33].

There is the potential for the production of a novel functional food—A fermented probiotic rice pudding that could be beneficial to people. The product could be a vehicle to deliver *L. rhamnosus* GR-1 to individuals with urogenital problems and urinary tract infections. This bacterium can help restore normal vaginal flora and can decrease side effects associated with antibiotic use [34–36]. In the near future, probiotic rice puddings could be consumed as part of a normal diet and thus contribute to the maintenance of good health. Apart from ensuring the viable cell count of probiotics for the duration of the rice puddings' shelf life, short-chain inulin, long-chain inulin, and oat in rice puddings can offer additional health benefits and nutritional value to the consumer. Various studies indicate that probiotic products fortified with inulin and oat are well received with panellists and that there is an interest in such a product [28,37,38].

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Author Contributions: Maja Williams performed the experiments, analysed the data and drafted this paper. Sharareh Hekmat designed the experiments, revised the manuscript and approved the final version for publication. All authors read and approved the final manuscript.

Conflicts of Interest: The authors declare no conflict of interest.

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