Synbiotic yogurt production by using prebiotic compounds and probiotic lactobacilli

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ABSTRACT: Synbiotic products contain both probiotic bacteria and prebiotic compounds. The aim of this study was to produce a synbiotic yogurt with desirable quality. Lactobacillus rhamnosus and Lactobacillus reuteri along with 1.5% prebiotics including inulin, lactulose, and oligofructose were used to make synbiotic yogurt (2.5% fat). The samples were stored at 4°C for 28 days, and their physicochemical, microbial, and sensory properties were investigated. The highest and the lowest pH values were observed for the samples containing lactulose and inulin, respectively. The lowest acidity was recorded for control and inulin-contained samples. The highest acidity was observed for the sample containing lactulose. The samples containing inulin and lactulose showed the lowest syneresis. The sample containing inulin had the highest viscosity. Lactobacillus rhamnosus and Lactobacillus reuteri, showed the best viability in the sample containing inulin. The sample containing oligofructose had the best taste followed by the sample containing inulin. Oligofructose- and inulin-contained samples had the best texture. The samples containing lactulose had the best smell followed by control and inulin-contained samples. The samples containing inulin and lactulose showed the most desirable sourness. The color of the samples showed no significant difference. Finally, the most acceptable samples were those which contained lactulose and inulin.

Keywords: Inulin; lactulose; Lactobacillus rhamnosus; Lactobacillus reuteri; oligofructose; yogurt

INTRODUCTION

Synbiotics consist of both probiotic microorganisms and prebiotic compounds. These products, in fact, contain useful bacteria (probiotics) and indigestible carbohydrates (prebiotics) for stimulating beneficial bacteria growth (Shi et al., 2002; Khurana and Kanawjia, 2007). Synbiotics have antimicrobial, anticancer, anti-allergic and immune-stimulating properties. They improve absorption of minerals, prevent incidence of diarrheah, and optimize assimilation of nutrients (Buterikis et al., 2008).

Probiotic bacteria along with prebiotics cause release of antibacterial substances such as bacteriocins, retarding growth of pathogenic bacteria (Kounav, 2007). Two bacterial species, Lactobacillus rhamnosus and Lactobacillus reuteri, are considered as probiotic agents with therapeutic properties (Guarner et al., 2008). Lactobacillus rhamnosus is one of important probiotic species for yogurt manufacture, preventing different infections due to bacteriocin production (Kingsley et al., 2008). The resistance of Lactobacillus rhamnosus to acids and vancomycine is considerable. Also, it has been established that Lactobacillus rhamnosus has anti-collitis and anti-cardiovascular disease effects as consumption of yogurt containing this bacterium play an effective role in reducing the incidence of cardiovascular diseases (Anukam et al., 2009). Lactobacillus reuteri is introduced as a probiotic in 1980. Initially it was important for producing a kind of antibiotic known as reuterin. A study conducted in 1986 showed that consumption of yogurt containing Lactobacillus reuteri reduced significantly intestinal coliforms count (Cadieux et al., 2008). Consumption of fermented dairy products containing Lactobacillus rhamnosus GR-1 and Lactobacillus reuteri GR-14 improve the function of brain in patients suffering from cerebrospinal disorder and urinary infection (Kingsley et al., 2008).

Prebiotic compounds include fructo-oligosaccharides (FOSs) such as inulin, galacto-oligosaccharides (GOSs), lactitol and lactulose, among others. FOSs especially inulin, lactulose, oligofructose, and lactitol are bifidos agents (Gibson et al., 2000; Lamoureux et al., 2002; Brunetti, 2007). Fructo-oligosaccharides (FOSs) are among the most important natural prebiotics. Inulin has β (1–2) binding with all its molecules terminate in glucose units. It consists of fructose components. Inulin may reduce blood sugar and cholesterol levels and improve bone health and calcium absorption when added to foods rich in calcium. In Australia as well as most countries, inulin has been added to different foods since 1990, resulting in improved mouthfeel, creaminess, reduced fat, improved texture and increased viscosity (especially in yogurt (Robertfroid et al., 1998; Kai, 2007; Kounga, 2007; Guarner, 2008). Oligofructose is among fructooligo-saccharides, consisting of glucose–β (1–2)–fructose, producing 1/5 Kcal/g. It is a dietary fiber and a prebiotic compound. It has a desirable sweet taste.
applied for food enrichment which helps in improving flavor and aroma, texture and sweet taste, having no negative effects. Its quality is similar to sugar or glucose syrup thus it is used as sugar or fat substitute for reducing calories of dairy products (Roberfroid et al., 1993; Franck and Coussement, 1997; Niness, 1999). Lactulose (β-D-0-4-galactopyranosyl–D-fructose) is C12H22O11 with a molecular mass of 342.3 g/mol (Matijevic et al., 2009).

The aim of this study was to produce a synbiotic yogurt containing Lactobacillus reuteri and Lactobacillus rhamnosus along with prebiotic compounds (inulin, oligofructose and lactulose).

**MATERIALS AND METHODS**

**Probiotic bacteria preparation**

Lyophilized stock cultures including Lactobacillus rhamnosus and Lactobacillus reuteri were obtained from Persian Type Culture Collection (PTCC). Active cultures were prepared from these stock cultures through two transfers in 10% (w/v) reconstituted skim milk (incubation at 37 °C for 24 h).

**Synbiotic yogurt production**

To produce probiotic yogurt, 1.5% (w/w) skim milk powder was added to 2.5% raw milk and heated (95°C, 15min). Then temperature decreased to 40-42°C and 1.5% prebiotics including inulin (medium chain, Sensus Co.), lactulose (Beneo Orafti Co.) and oligofructose (Sensus Co.) were individually added to the samples. The samples were inoculated with yogurt starters (Lactobacillus delbruckii subs. bulgaricus and Sterptococcus thermophillus) along with Lactobacillus rhamnosus and Lactobacillus reuteri as probiotics simultaneously. To make yogurt starters, a mixture of two types of starter with commercial formula Di-ROX TY 367 and Di-PROX TY 947 (DVS, DANISCO) in a ratio 1:1 with the value of 1% was added to milk samples. Moreover, Lactobacillus rhamnosus and Lactobacillus reuteri, each with the value of 2% (totally 4%) were added to milk samples. Count of probiotic lactobacilli was 108cfu/mL. Then the samples were incubated at 37°C until the pH reached about 4.7. The samples were cooled following two steps: One using dry gel in water to about 10-12°C and then in refrigerator to 4-6°C. The produced samples are shown in Table 1.

**Physicochemical analysis**

pH of the samples was measured using a pH meter (MA235, HANNA, Italy). Titratable acidity was determined by AOAC method (AOAC, 2002). The viscosity was measured with Brockfield viscometer (Brockfield Engineering Lab Inc., Stoughton, MA) using spindle No.4, RPM 25, at 8°C for 12 s in cp (Aryana, 2003). Syneresis was determined by centrifuging yogurt at 1200 ×g for 20 min (Keogh and Okenndey, 1998).

**Microbial analysis**

Enumeration of probiotic bacteria was used by Hekmat et al. method (Hekmat et al., 2009). MRS Agar containing fucidic acid (15μg/mL) for Lactobacillus rhamnosus and MRS Agar containing tetracycline (50μg/mL) for Lactobacillus reuteri using pour–plate method under anaerobic conditions (using anaerobic jar–gas pack system) was done. The samples were incubated at 37°C for 72 h.

**Sensory evaluation**

Sensory evaluation of synbiotic yogurt samples was performed by a trained panel (panelists) of PAK Factory. The given scores were designated as follows: 20-25, very good; 15-20, Good; 10-15, acceptable and >10, unacceptable, that the following were divided: Taste was designated totally 10 scores, texture = 6 scores, smell = 4 scores, sourness = 3 scores, and color =2 scores.

**Statistical analysis**

Experiments were performed in triplicate. The data was analyzed based on factorial model with completely randomized blocks using Duncan test. SPSS Software, version 18, was used for statistical analysis. To draw the diagrams, Excel software was used.

**RESULTS AND DISCUSSION**

**Physicochemical characteristics of synbiotic yogurt samples**

The results of measuring pH value of synbiotic yogurt samples are given in Table 2. Comparing means of treatments revealed that there was no significance difference between samples until 7th day (p>0.05). On 14 d, the sample containing oligofructose showed the highest pH value showing significant difference (p<0.05) from the sample containing inulin. Control sample and the sample containing lactulose did not show any significant difference (p>0.05) from the other samples. As shown in Table 2, variations trend of pH value was falling for all samples, and from 21 d pH decline was decelerated, however, most of samples did not show any
significant difference (p>0.05). It seems that addition of prebiotics rather than non-fat solid matter does not have any significant difference (p>0.05) on buffering capacity of the product during fermentation, because all treatments show similar pH decline rate. These results are similar to the findings of Hekmat et al. (2009), Ramchanrand and Shah (2010), and Mazloomi et al., (2011).

Acidity values of synbiotic yogurt samples during storage at 4°C are given in Table 3. As the results show (Table 3), acidity values of all samples follow a rising trend during 28 d of storage at 4°C. The sample containing inulin and the sample containing lactulose had the lowest and the highest acidity values, respectively. Variance analysis of the effect of treatment on acidity showed significance (p<0.05) on 1 and 21 d. Comparison of samples at 0 d revealed that the sample containing oligofructose had the highest acidity being significantly different (p<0.05) from control. There is no other significant difference (p>0.05). On 1 d, the highest mean was recorded for the sample containing lactulose and oligofructose showing a significant difference (p<0.05) from inulin contained sample as well as control. The highest mean was observed for the sample containing lactulose on 21 d, showing a significant difference (p<0.05) from control. Other samples did not show any significant difference (p>0.05). These results are similar to the findings of Antunes et al., (2005) and Oliverri et al., (2009), while they are not similar to results of Tabatabaie and Mortazavi (2008).

Syneresis values of synbiotic yogurt samples are presented in Table 4. The means obtained through syneresis tests were not significant (p>0.05). One reason for this decrease in syneresis of synbiotic yogurt is increase in texture consistency and water–complex capacity index allowing prebiotic compounds to prevent syneresis by increasing water–binding capacity (curve of syneresis percentage variations). Table 4 shows that initially the lowest syneresis value was observed for control sample and the highest value was recorded for oligofructose contained sample. The syneresis trend was falling until 21 d then it was rising again. At the end of storage period, the samples containing inulin and oligofructose showed the highest and the lowest syneresis respectively. On the whole the lowest syneresis was observed for inulin- and lactulose- contained samples and the highest value of syneresis was recorded for oligofructose- contained sample. These results are confirmed by the findings of Olson and Aryana (2008), Pasecphol (2008), and Tabatabaie and Mortazavi (2008).

The results of measuring viscosity of synbiotic yogurt during storage are given in Table 5. The effect of treatments on viscosity was significant (p<0.05) on 1, 7, 21 and 28 d (Table 5). Comparison of the treatments revealed that inulin- contained sample had the highest viscosity on 1 d being significantly different (p<0.05) from control, while it was not significantly different (p>0.05) from the samples containing lactulose and oligofructose. Also the samples containing lactulose and oligofructose were significantly different (p<0.05) from control. The lowest viscosity value was observed for control. On 7 d the highest mean was recorded for inulin-contained sample showing significant difference (p<0.05) from other samples. The viscosity of samples containing lactulose and oligofructose were significantly greater (p<0.05) than control. On 21 d, the highest mean was recorded for inulin-contained sample showing significant difference (p<0.05) from lactulose-contained and control samples. Also the viscosity of sample containing lactulose was significantly greater (p<0.05) than control. On 28 d, the sample containing inulin had the highest mean value being significantly different (p<0.05) from control. The remaining samples showed no significant difference (p>0.05). Walistra et al., (2006) stated that firmness of set yogurt decreases as pH value increases, thus, considering pH decline in this study an increase in viscosity is justified.

Microbial count of synbiotic yogurt samples

The results of Lactobacillus rhamnosus count in the samples during storage are presented in Table 6. Comparing treatments revealed that significant difference (p<0.05) between samples on 1, 7, 21 and 28 d (Table 6). On 1 d, the sample containing inulin had the highest mean being significantly different (p<0.05) from remaining samples. Oligofructose-contained sample was significantly (p<0.05) greater than the sample containing lactulose. Other samples did not show any significant difference (p>0.05). On 7 d, inulin-contained sample had the highest mean showing significant difference (p<0.05) from other samples. Control sample was significantly (p<0.05) greater than lactulose- contained sample. The remaining samples showed no significant difference (p>0.05). On 21 d, the sample containing inulin had the highest mean value with significant difference (p<0.05) from other samples. The samples containing lactulose and oligofructose were significantly (p<0.05) greater than control. On 28 d, the highest mean was recorded for the sample containing inulin showing significant difference (p<0.05) from control sample. Also the samples containing lactulose and oligofructose were significantly greater than control sample (p<0.05). In all samples, Lactobacillus rhamnosus count was higher than standard of 106 (IDF/FIL) till the end of 28 d.

The results of Lactobacillus reuteri count during storage are given in Table 7. As shown in Table 7, there was no significant difference between the samples (p>0.05). In all samples Lactobacillus reuteri count was higher than standard of 106 (IDF/FIL) until the end of 21 d. The study showed that Lactobacillus reuteri and Lactobacillus rhamnosus could be added to milk as carriers. These are well – known probiotic species providing desirable sensory and therapeutic properties in dairy products as well as consumers (Hekmat et al., 2009). Milk- based products are favorable environment for probiotics to grow. Standard of probiotic products
specifies that minimum acceptable level of probiotics in dairy products should be 10^6 - 10^7 cfu/gr being able to provide therapeutic properties, while daily consumption of these products must be minimum 100 ml or 100g (Terri et al., 2004). The results are similar to the findings of Mohebbi and Ghoddusi (2008) on the effect of lactulose and inulin on stimulating probiotics growth.

**Sensory characteristics of synbiotic yogurt samples**

The scores designated for taste of synbiotic yogurt samples are presented in Figure 1. The effect of treatment on taste of samples was significant only on 21 d (p<0.05). Comparing means of treatments showed that the sample containing oligofructose was significantly higher in score than lactulose- and inulin-contained samples (p<0.05). Other samples did not show any significant difference (p>0.05).

The results of measuring texture of synbiotic yogurt samples during storage are given in Figure 2. Comparing treatments revealed that the effect of treatments on texture was only significant (p<0.05) on 7 d (Figure 2). The highest mean was recorded for the sample containing lactulose being significantly different (p<0.05) from control sample. Control sample was significantly lower (p<0.05) in score than other samples. The remaining samples did not show any significant difference (p>0.05). Also, inulin- contained sample was significantly higher (p<0.05) in score than control. Other samples showed no significant difference (p>0.05).

The results of measuring smell of synbiotic yogurt samples are given in Figure 3. Smell index ranged 1.91< S <3 during storage (Figure 3). All samples did not show any significant difference (p>0.05).

The results of measuring sourness of synbiotic yogurt samples are shown in Figure 4. Sourness index ranged 1.25< S<2.50 during storage.

The results of measuring color of synbiotic yogurt samples: All samples achieved totally score (2 scores).

The results of total acceptability of synbiotic yogurt samples during storage are given in Figure 5. The effect of treatments on total acceptability of samples was significant (p<0.05) only on 7 d. The highest mean was recorded for inulin–contained sample, being significantly greater (p<0.05) than control sample. The remaining samples did not show any significant difference (p>0.05). Total acceptability ranged 12.18<A<17.25 during storage (Figure 5). On the whole, the most acceptable samples were those containing inulin and lactulose.

It has been reported that addition of prebiotics such as lactulose and inulin and / or a mixture of these two compounds especially in dairy products cause an increase in consistency and acceptability and may improve mouthfeel (Golob et al., 2004). This corresponds to our results. Some researchers have suggested that sensory properties of probiotic yogurt are lost during storage as compared to ordinary counterpart unless stabilizers or prebiotic compounds are added (Hekmat and Reid, 2006). In one study, sensory attributes of probiotic yogurt along with control one were investigated. The results showed that total acceptability of 1% fat- contained probiotic yogurt was similar to of 1% fat- contained control (Hekmat and Reid, 2006). These results confirm the findings obtained by Khurana and Kanawjia (2007) and Cruz et al, (2010), while they are not similar to findings of Hekmat and Reid (2006), Donkor (2007), Matijevic et al., (2009).
Table 3. Acidity (ºD) of synbiotic yogurt samples during 28 d of storage at 4ºC (Mean ± SD)

<table>
<thead>
<tr>
<th>Day</th>
<th>Sample</th>
<th>Acidity (ºD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>C</td>
<td>75.33±0.333b</td>
</tr>
<tr>
<td>1</td>
<td>C</td>
<td>81.00±0.577 a</td>
</tr>
<tr>
<td>7</td>
<td>C</td>
<td>83.67±0.882 a</td>
</tr>
<tr>
<td>14</td>
<td>C</td>
<td>87.33±1.667b</td>
</tr>
<tr>
<td>21</td>
<td>C</td>
<td>92.33±1.202 a</td>
</tr>
</tbody>
</table>

Table 4. Syneresis values (%) of synbiotic yogurt samples during 28 d of storage at 4ºC (Mean ± SD)

<table>
<thead>
<tr>
<th>Day</th>
<th>Sample</th>
<th>Syneresis (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>C</td>
<td>27.22±1.380 a</td>
</tr>
<tr>
<td>1</td>
<td>C</td>
<td>27.86±0.776 a</td>
</tr>
<tr>
<td>7</td>
<td>C</td>
<td>28.89±0.627 a</td>
</tr>
<tr>
<td>14</td>
<td>C</td>
<td>26.19±0.787 a</td>
</tr>
<tr>
<td>21</td>
<td>C</td>
<td>27.27±1.746 a</td>
</tr>
</tbody>
</table>

Table 5. Viscosity (cp) values of synbiotic yogurt samples during 28 d of storage at 4ºC (Mean ± SD)

<table>
<thead>
<tr>
<th>Day</th>
<th>Sample</th>
<th>Viscosity (cp)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>C</td>
<td>21.23±0.060b</td>
</tr>
<tr>
<td>1</td>
<td>C</td>
<td>21.76±0.069c</td>
</tr>
<tr>
<td>7</td>
<td>C</td>
<td>22.44±0.233 a</td>
</tr>
<tr>
<td>14</td>
<td>C</td>
<td>23.07±0.091c</td>
</tr>
<tr>
<td>21</td>
<td>C</td>
<td>23.04±0.088b</td>
</tr>
</tbody>
</table>

Table 6. Lactobacillus rhamnosus (log cfu/ml) count in synbiotic yogurt samples during 28 d of storage at 4ºC (SD ± mean)

<table>
<thead>
<tr>
<th>Day</th>
<th>Sample</th>
<th>Lactobacillus rhamnosus (log cfu/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>C</td>
<td>8.51±0.162bc</td>
</tr>
<tr>
<td>1</td>
<td>C</td>
<td>8.26±0.160b</td>
</tr>
<tr>
<td>7</td>
<td>C</td>
<td>7.42±0.187 a</td>
</tr>
<tr>
<td>14</td>
<td>C</td>
<td>6.39±0.353c</td>
</tr>
<tr>
<td>21</td>
<td>C</td>
<td>1.00±1.880b</td>
</tr>
</tbody>
</table>

Table 7. Lactobacillus reuteri (log cfu/ml) count in synbiotic yogurt samples during 28 d of storage at 4ºC (SD ± mean)

<table>
<thead>
<tr>
<th>Day</th>
<th>Sample</th>
<th>Lactobacillus reuteri (log cfu/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>C</td>
<td>8.13±0.134a</td>
</tr>
<tr>
<td>1</td>
<td>C</td>
<td>7.88±0.110a</td>
</tr>
<tr>
<td>7</td>
<td>C</td>
<td>7.09±0.272a</td>
</tr>
<tr>
<td>14</td>
<td>C</td>
<td>3.65±1.936 a</td>
</tr>
<tr>
<td>21</td>
<td>C</td>
<td>1.000</td>
</tr>
</tbody>
</table>

* Similar letters represent non-significance (p>0.05)
Figure 1. Curve of taste variations of synbiotic yogurt samples during storage at 4°C.

Figure 2. Curve of texture variations of synbiotic yogurt samples during storage at 4°C.

Figure 3. Curve of smell variations of synbiotic yogurt samples during storage at 4°C.
Figure 4. Curve of sourness variations of synbiotic yogurt samples during storage at 4°C.

Figure 5. Curve of total acceptability variations of synbiotic yogurt samples during storage at 4°C.

CONCLUSION

High quality synbiotic yogurt was made using Lactobacillus rhamnosus and Lactobacillus reuteri along with prebiotics including inulin, lactulose and oligofructose. Inulin with a value of 1.5% had more pronounced positive effect on probiotics survival especially Lactobacillus rhamnosus as well as on quality of synbiotic yogurt compared with other prebiotic compounds. However, other prebiotics produced similar results.

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