INT. J. BIOL. BIOTECH., 9 (4): 359-365, 2012.

INVESTIGATION OF VIABILITY OF PROBIOTIC BACTERIA AND PHYSICO-CHEMICAL CHARACTERISTICS OF SYNBIOTIC YOGURT DURING COLD STORAGE

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ABSTRACT

Consuming synbiotic products (simultaneous presence of probiotics and prebiotics) has beneficial effects on health of consumers. The aim of this study was to determine the effect of inulin concentration (1.5, 2 and 2.5%) and kind of probiotic culture (*Lactobacillus casei* and *Lactobacillus acidophilus* separately, and mixture of them) on physicochemical, microbial and sensory characteristics of synbiotic yogurt. Sensory evaluation, determination of pH, acidity, syneresis and viscosity, and number of probiotic bacteria in all the samples were conducted on the first day after production. The samples with better sensory quality were stored in 4 °C for three weeks and all analyses were conducted in days 7, 14 and 21. During cold storage, pH of the samples decreased significantly ($p<0.05$) whereas acidity and viscosity increased significantly ($p<0.05$). Syneresis in sample containing mixed probiotic bacteria and 2% inulin did not change until 14th day then increased significantly ($p<0.05$). Syneresis in sample containing *L.acidophilus* and 2% inulin and sample containing *L.casei* and 1.5% inulin increased significantly ($p<0.05$) until 7th day but after that decreased. Number of probiotic bacteria increased significantly ($p<0.05$) until 7th day then decreased, but this decrease was not significant.

Keywords: Inulin, *Lactobacillus acidophilus*, *Lactobacillus casei*, Physicochemical characteristics, Sensory evaluation, Synbiotic yogurt

INTRODUCTION

Functional food products have shown the high potential of growth in the recent years, there has been a growing interest in using probiotic bacteria as dietary adjuncts in the dairy industry (Kailasapathy and Sultana, 2003). In the attempt to obtain products with benefits to the consumer health, probiotics as well as a certain number of prebiotics such as soluble fibers are added to functional dairy products (Mattila-Sandholm *et al*., 2002). Most common probiotics used in functional dairy products belong to the genera of Lactobacillus and Bifidobacterium, (Goldin and Gorbach, 1984). The efficacy of probiotic bacteria depends on their counts. Their survival must be maintained throughout the product's shelf-life and they must survive in the gut environment. To exert positive health effects, they have to establish themselves in certain numbers in the gastrointestinal tract (Kailasapathy and Sultana, 2003). A standard, requiring a minimum of 10^6–10^7 cfu/mL of *L. acidophilus* and/or bifidobacteria in fermented milk products, has been introduced by several food organizations world-wide (IDF, 1992). Prebiotics are defined as non-digestible carbohydrates that benefit their hosts by selective stimulation of growth or viability of one or limited number of bacteria in gut. Consuming synbiotic products (simultaneous presence of probiotics and prebiotics) has more beneficial effects on health of consumer (Gonzalez-Martinez *et al*., 2002). Inulin is one of the most of prebiotic compounds. This food ingredient is a soluble and fermentable fiber named fructan, which cannot be digested by amylase or other hydrolytic enzymes in the upper section of the intestinal tract (Carabin and Flamm, 1999). Inulin was employed in some studies mainly devoted to increase the viability of probiotic microorganisms during long-term cold storage of probiotic milk products (Akalin *et al*., 2004; Bruno *et al*., 2002; Shin *et al*., 2000).

Moreover, it can stimulate the probiotic bacteria without adversely affecting flavor of product (El-Nagar *et al*., 2002; O¨ Zer *et al*., 2005). The aim of this study was to determine the effect of inulin concentration and kind of probiotic culture on physicochemical, microbial and sensory characteristics of synbiotic yogurt.

MATERIALS AND METHODS

1. Milk preparation

Raw milk was prepared from Choopan Dairy Company (Varamin, Iran). Milk was standardized with 2.5% fat and 8.6% solids not fat.

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2. Starter cultures

The DVS pouches of commercial lyophilized cultures including yogurt starter YC-X11 (mixed culture of *Lactobacillus delbrueckii* ssp. *bulgaricus* and *Streptococcus thermophilus*) and pure strains of *Lactobacillus acidophilus* (LA-5) and *Lactobacillus casei* (LC-01) were supplied by Chr-Hansen (Denmark). The bag of yogurt culture was solved in 500 mL sterile reconstituted milk (prepared with 10% skim milk powder). The packages of *Lactobacillus casei* and *L.acidophillus* were solved in 500 mL sterile reconstituted milk separately.

3. Synbiotic yogurt manufacture

Milk samples were heated at 85 °C for 30 min then cooled down to 40 °C for inoculation. Different concentrations (1.5%, 2% and 2.5%) of medium-chain inulin (Orafti, Malvern) were added to milk samples.

All the samples were inoculated with 1.5% yogurt culture and 1% probiotic culture (*L.casei* and *L.acidophilus* separately, and mixture of them). Inoculated samples were poured in 500 g plastic cups with snap on lids and incubated at 40 °C until the pH dropped to 4.6. Then the samples were kept at 4 °C. At the second part of experiments, the samples with the most general acceptability were selected. Physicochemical characteristics and viability of probiotic bacteria in these samples were evaluated during 21-days of refrigerated storage.

4. Physicochemical analysis

pH of the samples was measured using a pH meter (MA235, HANNA, Milan, Italy). Titratable acidity was determined by AOAC method (AOAC, 2002).Viscosity was measureded by a Brookfield DV II+ viscometer (Brookfield Engineering Lab Inc, Stoughton, MA) at 8 °C according to Aryana Method (Aryana, 2003). Syneresis was determined by centrifuging yogurt at 350 G for 30 min and it was expressed as volume of separated whey per 100 ml of yogurt (Gonzalez-Martinez et al., 2002).

5. Microbiological analysis

MRS-bile agar (MRS agar from Merck, Germany and bile from Sigma, USA) was used for the selective enumeration of probiotic bacteria in the presence of yogurt bacteria. The plates were incubated anaerobically at 37°C for at least 72 h (Tharmaraj, 2003).

6. Sensory evaluation

Sensory evaluation was conducted using a seven member experienced panel. The sensory attributes were flavor, texture and color. A 1–4 point scale was used (Aryana, 2003). The acceptability values were scored on 4 (very good), 3 (good), 2 (moderate), and 1 (bad).

7. Statistical analysis

Experiments were performed in triplicate. Data were analyzed by analysis of variance using Proc Mixed of the Statistical Analysis Systems (SAS, 2004). Significant differences between means were determined using Fisher's protected Least Significant Difference test.

RESULTS AND DISCUSSION

1.Evaluation of synbiotic yogurt samples one day after the production

1.1 Physicochemical characteristics

Table 1 demonstrates physicochemical characteristics of synbiotic yogurts one day after the production. The lowest pH was for sample containing *L.casei* and 1.5% inulin. The sample containing *L.casei* and 2.5% inulin had the highest pH. The lowest acidity was for sample containing *L.acidophilus* and 2% inulin. The sample containing *L.casei* and 2.5% inulin had the highest acidity. There is a significant difference between samples ($p<0.05$). Paseephol (2008) reported that the addition of inulin powders regardless of the type used didn't affect the initial pH and acidity of yogurt and showed that the low level of post-acidification in these yogurt be attributed to the type of probiotic and yogurt starters used.

The highest viscosity was for sample containing *L.casei* and 1.5% inulin. The sample containing *L.acidophillus* and 2.5% inulin had the lowest viscosity.There is a significant difference between samples ($p<0.05$). Niness (1999) reported that both inulin and oligofructose add fiber without contributing to the viscosity of product.

The highest syneresis value was for sample containing *L.casei* and 2.5% inulin. The sample containing *L.casei* and 2% inulin had the lowest syneresis. There is a significant difference between samples ($p<0.05$).Addition of inulin and FOS to set yogurt caused significant decrease in synersis (Nastaj and Gustaw, 2008). Similary, Guven et al. (2005) investigated the possibility of using different concentrations (1%, 2% and 3%) of long-chain inulin as fat replacer in low-fat yogurt. They found that whey separation and consistency increased with inulin concentration.

Table 1.Physicochemical characteristics of synbiotic yogurt samples one day after production (values are means ± SD)

Sample	Inulin	Probiotic culture	pH	Acidity (°D)	Viscosity (cp)	Syneresis (%)
AC1	1.5	LA-5	4.52±0.04(ab)	66.66±0.57(bcd)	12.07±0.72(d)	6.10±0.70(a)
AC2	2	LA-5	4.52±0.02(ab)	64.33±1.52(d)	12.27±0.28(d)	5.01±0.52(ab)
AC3	2.5	LA-5	4.47±0.05(bc)	70.00 ±3.00 (b)	8.65±0.26(e)	6.17±0.66(a)
CA1	1.5	LC-01	4.45±0.02(c)	66.00± 1.15(cd)	16.68±0.39(a)	2.70 ±1.03(c)
CA2	2	LC-01	4.51±0.06(ab)	67.66±2.51(bcd)	15.79±0.56(ab)	2.64±1.19(c)
CA3	2.5	LC-01	4.54±0.05(a)	74.33±1.52(a)	16.26±0.47(ab)	4.30±1.22(abc)
M1	1.5	LA-5 + LC-01	4.52±0.07(ab)	70.00 ±2.28(b)	14.23±0.29(c)	3.13±0.96(bc)
M2	2	LA-5 + LC-01	4.52±0.02(ab)	66.66±1.73(bcd)	15.73±0.27(ab)	5.14±0.63(ab)
M3	2.5	LA-5 + LC-01	4.50±0.04(b)	68.33±2.08(bc)	15.40±0.25(b)	5.51±1.12(ab)

Values in the same column shown with similar letters are not significantly different

1.2 Microbial characteristics

Number of probiotic bacteria in the samples one day after production is presented in Table 2. The highest probiotic number was for sample containing *L.acidophillus* and 2.5% inulin. There is no significant difference between samples (p>0.05) except sample containing *L.acidophillus* and 2.5% inulin. Number of probiotic bacteria in this sample was above 7 log cfu/mL. The viability of lactic acid bacteria in fermented milk can be increased by inulin. Sadek et al. (2004) reported that growth of *Lactobacillus acidophilus* and *Lactobacillus rhamnosus* was enhanced with 2% inulin.

Table 2. Counts of probiotic bacteria in the samples one day after production

Sample	Inulin	Probiotic culture	Number of probiotic bacteria (log cfu/mL)
AC1	1.5	LA-5	6.25±0.27(b)
AC2	2	LA-5	6.24±0.40 (b)
AC3	2.5	LA-5	7.36±0.11(a)
CA1	1.5	LC-01	6.07±0.81(b)
CA2	2	LC-01	6.44±0.46(b)
CA3	2.5	LC-01	6.15±0.13(b)
M1	1.5	LA-5 + LC-01	6.53±0.40 (b)
M2	2	LA-5 + LC-01	6.37±0.35(b)
M3	2.5	LA-5 + LC-01	6.67±0.62(b)

Values in the same column shown with similar letters are not significantly different.

Table 3.Sensory properties of synbiotic yogurt samples one day after produc (values are means ± SD)

Sample	Inulin	Probiotic culture	Flavor	Texture	Color
AC1	1.5	LA-5	1.5±0.67(de)	1.16±0.38(c)	3.58±0.51(a)
AC2	2	LA-5	3.25±0.75(a)	1.50±0.67(bc)	3.75±0.45(a)
AC3	2.5	LA-5	3.08±0.9(ab)	1.33±0.65(c)	3.75±0.45(a)
CA1	1.5	LC-01	1.58±0.9(cde)	2.5±0.67(a)	3.58±0.51(a)
CA2	2	LC-01	2.41±0.79(abcd)	2±0.85(abc)	3.58±0.51(a)
CA3	2.5	LC-01	2±0.85(cde)	2.33±0.77(ab)	3.58±0.51(a)
M1	1.5	LA-5 + LC-01	2.5±1.16(abc)	2.33±0.99 (ab)	3.50±0.67(a)
M2	2	LA-5 + LC-01	2.16±0.71(bcde)	2.41±0.66(a)	3.41±0.51(a)
M3	2.5	LA-5 + LC-01	1.25±0.45(e)	2.50±1.00 (a)	3.66±0.65(a)

Values in the same column shown with similar letters are not significantly different.
The scores of color of the samples were not different significantly (p>0.05).

1.3 Sensory characteristics

Table 3 demonstrates sensory characteristics of the samples one day after production. The highest flavor score was for sample containing *L.acidophillus* and 2% inulin (AC2). The sample containing mixed probiotic culture and 2.5% inulin (M3) had the lowest flavor score. There is a significant difference between samples ($p<0.05$).

The samples containing mixed probiotic culture and 2.5% inulin (M3), L.casei and 1.5% inulin (CA1), mixed probiotic culture and 2% inulin (M2) had the highest texture score ($p<0.05$).

Based on experiments in this stage, samples containing *L.acidophillus* and 2% inulin (AC2), *L.casei* and 1.5% inulin (CA1), and mixed probiotic culture and 2% inulin (M2) had the best organoleptic quality. These samples were stored at 4°C for three weeks.

2. Evaluation of the best samples (AC2, CA1 and M2) during cold storage

2.1. pH

The values of pH in synbiotic yogurt samples are shown in Figure **1**. pH of the samples decreased significantly ($p<0.05$) during the storage period. There was no significant difference between pH of the sample containing *L.casei* and 1.5% inulin (CA1) on 14th and 21th days. Moreover, there was no significant difference between pH of samples AC2 (containing *L.acidophilus* and 2% inulin) and M2 (containing mixed probiotic bacteria and 2% inulin) on 7th and 14th days. Similar results for pH values were observed for commercial yogurts containing probiotics during their storage (Shah et al., 2000). Hauly et al. (2005) manufactured soy yogurt with inulin and reported a decline in pH with storage time. The pH in 14th day was ($p<0.05$) lower than the other days.

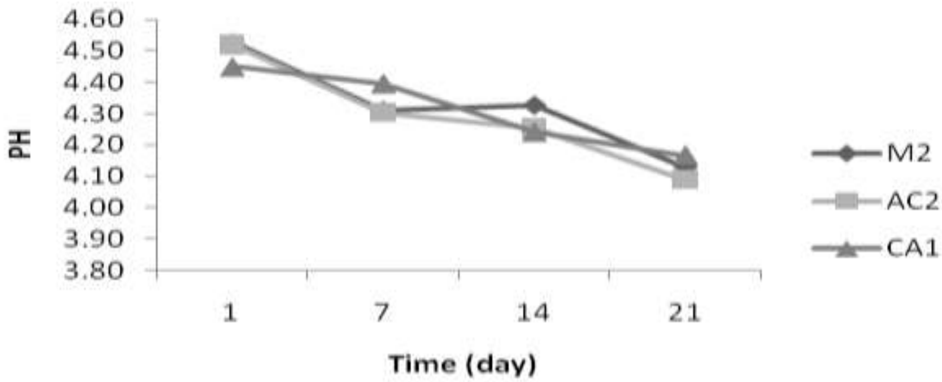

Fig. 1. Changes of pH in synbiotic yogurt samples (CA1, AC2 and M2) during refrigerated storage

2.2. Acidity

The acidity values are shown in figure **2**. Acidity of these samples increased significantly ($p<0.05$) during cold storage. There was no significant difference between acidity of the samples CA1, AC2 and M2 on 7th and 14th days. Similary, Ozer et al. (2005) reported an increase in acidity of probiotic yogurt containing inulin and lactulose during refrigerated storage.

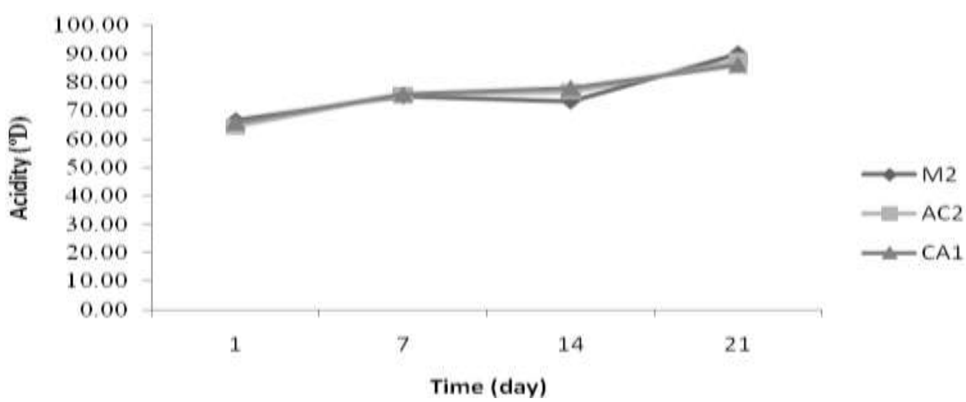

Fig. 2.Changes of acidity in synbiotic yogurt samples (CA1, AC2 and M2) during refrigerated storage

2.3 Viscosity

The viscosity values of synbiotic yogurt samples are reported in Figure **3**.The viscosity increased significantly ($p<0.05$) with time for all the samples. There was no significant difference between viscosity of the samples on 14th and 21th days. Kip et al. (2006) found that the addition of inulin with different chain length at different concentrations (1.5%, 3%, and 4%) to low-fat yogurts increased viscosity and this increase was higher when long-chain inulin was used.

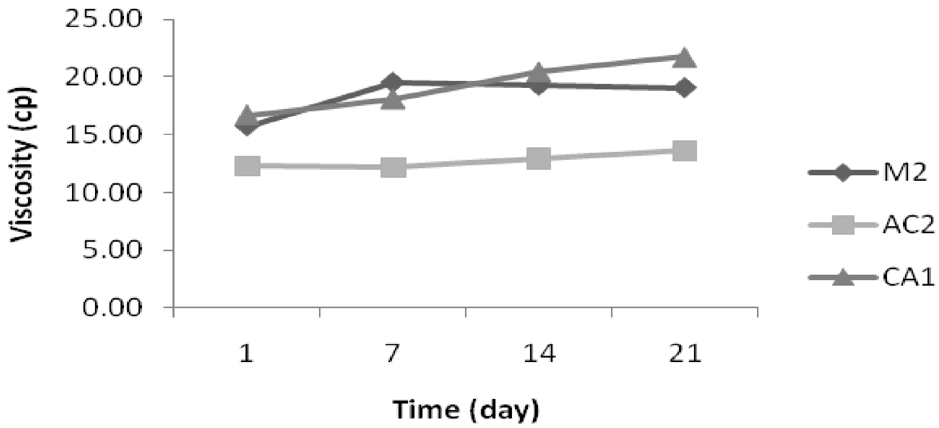

Fig. 3.Changes of viscosity in synbiotic yogurt samples (CA1, AC2 and M2) during refrigerated storage

2.4 Syneresis

The syneresis (released serum) values of synbiotic yogurt samples are presented in Figure **4**. There was not a steady rate in syneresis in these samples with an increase in storage time. Syneresis in sample M2 (containing mixed probiotic bacteria and 2% inulin) did not change until 14th day but after that increased significantly ($p<0.05$). Syneresis in samples AC2 (containing *L.acidophilus* and 2% inulin) and CA1 (containing *L.casei* and 1.5% inulin) increased significantly ($p<0.05$) until 7th day but after that decreased. Reduced syneresis of probiotic yogurt containing *L.casei* in the presence of lactolose-inulin has been reported in another study (Paseephol, 2008).

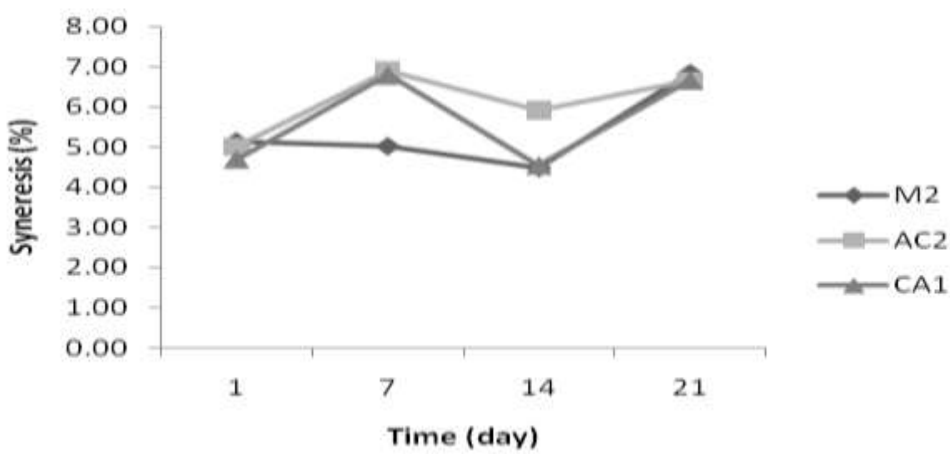

Fig. 4. Changes of syneresis in synbiotic yogurt samples (CA1, AC2 and M2) during refrigerated storage

2.4 Survival of probiotic bacteria

The counts of probiotic bacteria in synbiotic yogurt samples are reported in Fig. **5**. Number of probiotics in all samples increased significantly ($p<0.05$) until 7th day. After that the counts declined with time but this decrease was not significant. These results are similar to findings of Akalin et al. (2004) who reported higher counts of *Bifidobacterium longum* and *Bifidobacterium animalis* in yogurts containing fructooligosaccharide compared to yogurts without this prebiotic. They further reported declining counts of bifidobacteria over 28 d storage at 4°C. Shin et al. (2000) reported a decrease in mean doubling time and increase in viability of Bifidobacterium spp. in skim milk with increasing concentration of inulin and oligosaccharides up to a maximum of 50 g/L.

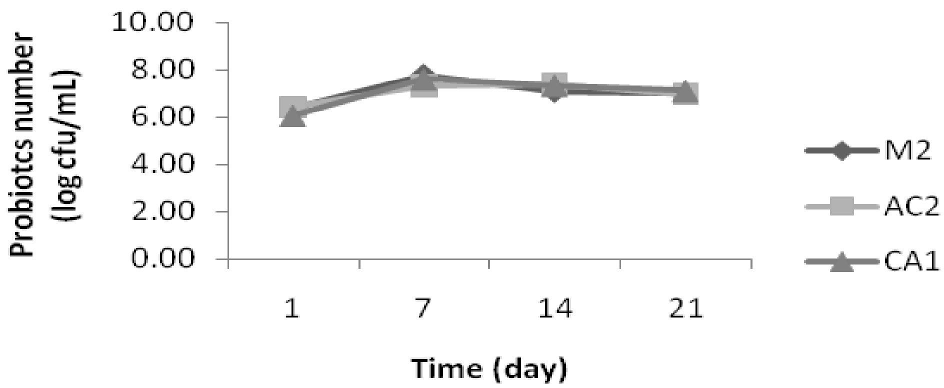

Fig. 5. Changes of number of probiotic bacteria in synbiotic yogurt samples (CA1, AC2 and M2) during refrigerated storage

Conclusion

Kind of probiotic culture and concentration of inulin affected significantly ($p<0.05$) pH, acidity, viscosity, syneresis, counts of probiotics, flavor and texture of synbiotic yogurt. During cold storage, pH of the samples decreased significantly ($p<0.05$) whereas acidity and viscosity increased significantly ($p<0.05$). Syneresis in sample containing mixed probiotic bacteria and 2% inulin did not change until 14th day then increased significantly ($p<0.05$). Syneresis in sample containing *L.acidophilus* and 2% inulin and sample containing *L.casei* and 1.5% inulin increased significantly ($p<0.05$) until 7th day but after that decreased. Number of probiotic bacteria increased significantly ($p<0.05$) until 7th day then decreased, but this decrease was not significant.

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(*Accepted for publication October 2012*)