Effect of adding inulin on microbial and physico-chemical properties of low fat probiotic yogurt

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Summary

Currently, due to their beneficial effects, there is interest in adding prebiotics to food products. This study investigated the effect of the addition of inulin (1% and 2%) on microbial and physico-chemical properties of probiotic low fat yogurt manufactured with *Lactobacillus acidophilus*. Six experimental preparations of yogurt were produced. Homogenized, standardized and pasteurized low fat milk were divided into six portions. Four portions were fortified with 1% and 2% inulin and two portions were used without inulin. All of the preparations were heated up to 85°C and fermented at 42°C until a pH of 4.6 was reached. Titratable acidity and pH were determined during the incubation period of the samples and a storage time up to 14 days. Syneresis, color, sensory evaluation and bacterial counts were determined during the storage time. The results showed that inulin did not significantly affect the titratable acidity and pH of the yogurts after 4 h of fermentation at 40°C. There were no significant differences between pH, titratable acidity, syneresis, color and sensory evaluation of all treatments on days 1, 7 and 14 of storage. The counts of *L. acidophilus* and *L. delbrueckii* ssp. *bulgaricus* declined over time, but the addition of inulin to the milk increased the viability of these bacteria during the storage of synbiotic yogurt. In conclusion, inulin can be used to manufacture low fat synbiotic yogurt with additional nutritional benefits without affecting the physico-chemical properties of yogurt.

Key words: Probiotic, Synbiotic, Yogurt, Inulin, *Lactobacillus acidophilus*

Introduction

As nutrition is moving towards the use of foods to promote better health and well-being, functional foods such as probiotic products have become more important to consumers (Cardarelli *et al*., 2008). The presence of probiotics in food has been associated with several health benefits including improvement in lactose metabolism, antimutagenic properties, anticarcinogenic activities, reduction in serum cholesterol level, anti-diarrheal properties, and suppression of *Helicobacter pylori* infection to name only a few (Shah, 2007).

Prebiotics are defined as "selectively fermented ingredients that allow specific changes, both in the composition and/or activity in the gastrointestinal microflora that confer benefits on host well-being and health" (Gibson *et al*., 2004). Currently, there is interest in the addition of inulin and oligofructose to food products (e.g., yogurt) for their beneficial effects (e.g., promoting lactobacilli and bifidobacterial growth in the colon, improving bioavailability of a variety of minerals such as calcium and iron and enhancing immune functions).

If both probiotics and prebiotics are combined together in a food product, it is called symbiotic, and presumably can impart the beneficial effects of both ingredients (Roberfroid, 2000). The objective of this study was to investigate the effect of the addition of inulin on the pH changes and decrease in acidity during the incubation period and on physico-chemical characteristics and bacterial counts of probiotic yogurt during storage at 4°C for 14 days.

Materials and Methods

Lactobacillus acidophilus LA-5 and commercial yogurt culture YC-288 containing *Streptococcus thermophilus* and *L. delbrueckii* ssp. *bulgaricus* (Chr. Hansen, Hørsholm, Denmark) were prepared. High-performance inulin (Beneo^{TM} HP) with an average degree of polymerization of ≥23 was kindly gifted from DKSH, London, UK.

Preparation of yogurt

Six experimental preparations of yogurt including control plain yogurt (T1), yogurt containing 1% inulin (T2), yogurt containing 2% inulin (T3), probiotic yogurt containing *L. acidophilus* (T4), synbiotic yogurt containing *L. acidophilus* plus 1% inulin (T5), and synbiotic yogurt containing *L. acidophilus* plus 2% inulin (T6) were prepared according to Akalin *et al*. (2007) and Aryana *et al*. (2007), with some modifications. All yogurt samples were produced in hygienic conditions.

Homogenized, standardized and pasteurized milk (3.62% protein, 3.61% lactose, 1.6% fat and 9.70% total solid) were divided into six equal portions (T1-T6), of which, four (T1-T4) portions were fortified with 1% and 2% inulin. All the preparations were heated up to 85°C for 30 min followed by cooling down to 40°C. The yogurt starter culture was then added at a concentration of 0.05% w/v to the T1-T3 samples. T4-T6 samples were prepared by adding yogurt starter culture (0.05% w/v) plus *L. acidophilus LA-5* (0.05% w/v). The mixtures were subsequently poured into 250-mL plastic cups and incubated at 40°C until their pH decreased to 4.6; they were then stored at 4 ± 1°C for 14 days. The entire experiment was replicated three times.

Determination of pH and titratable acidity

pH of the milk and yogurt samples was determined with a Methrohm pH meter 605 (Methrohm AG, Herisau, Switzerland) at room temperature. pH was determined in a single cup of yogurt per replication 1, 2, 3, and 4 h after inoculation, and in three cups of yogurt per replication 1, 7 and 14 days of storage.

Titratable acidity was determined in milk and yogurt samples at room temperature according to the methods described in AOAC (1999). Yogurt samples (10 g) were diluted with 10 mL distilled water and titrated with 0.1 M NaOH in the presence of phenolphthalein. Titratable acidity was expressed as the percent of lactic acid based on the sample weight. Titratable acidity was determined in a single cup of yogurt per replication 1, 2, 3 and 4 h after inoculation, and in three cups of yogurt per replication 1, 7 and 14 days of storage.

Color

The color parameters *L** (lightness-darkness), *a** (red-green axis) and *b** (yellow-blue axis) values of the yogurt samples were determined by the modified method described by Yam and Papadakis (2004) at 8°C. High resolution photos of the samples were taken by a digital camera. The photos were then analyzed by Adobe Photoshop for Windows® ver 8. Colorimetric values were determined in the Lab mode of the software. Color was determined in three cups of yogurt per replication 1, 7 and 14 days of storage.

Syneresis

The released whey in the yogurt samples was measured according to Aryana *et al*. (2007) by inverting a 50-g sample at 25°C on a fine mesh screen (Aperture 38 µm) placed on top of a funnel. The quantity of whey collected after 1 h of drainage at room temperature (25°C) was used as an index of syneresis. Syneresis was determined for three cups of yogurt per replication 1, 7 and 14 days of storage.

Sensory evaluation

Sensory evaluation was conducted by 30 untrained panelists. Samples were provided to panelists 1, 7 and 14 days of storage, in a random order using identical containers coded with random numbers. The flavor, texture, mouth-feel and color of the samples were evaluated on a four-point scale, with score 4 indicating “excellent” and score 1 representing “poor,” according to the Iranian National Standards (2002). The total score was calculated as the sum of scores of all the attributes.

Bacterial counts

The number of *L. acidophilus* in probiotic and synbiotic yogurts was counted according to Tharmaraj and Shah (2003). Inoculated plates in duplicates were incubated anaerobically using gas-generating pack A (Merck, Darmstadt, Germany) at 37°C for 72 h. Rough, dull, small, brownish colonies of 0.1-0.5 mm in diameter were counted.

Streptococcus thermophilus was enumerated according to the method described by Dave and Shah (1996). The inoculated ST agar plates in duplicates were incubated aerobically at 37°C for 24 h.

MRS agar (Merck) was adjusted to pH = 4.6, and anaerobic incubation (using gas-generating pack A, Merck) at 43°C for 72 h were used for differential enumeration of *L. delbrueckii* ssp. *bulgaricus* (Tharmaraj and Shah, 2003). White, cottony, rough, irregular colonies of 1.0 mm in diameter were counted.

Statistical analysis

The data were analyzed using SPSS for Windows® ver 13.0 (SPSS Inc., Chicago, IL, USA). One-way ANOVA, Scheffe’s and Kruskal-Wallis tests were used. A p-value <0.05 was considered as the significant level for all tests.

Results

The pH values are shown in Table 1. Those were between 4.55 and 4.94 after 4 h of fermentation. Although no statistically significant difference was observed in the pH of the treatment groups, the pH was lower in T6 after 4 h of fermentation. The pH of the yogurt samples which contained inulin was not significantly different on days 1, 7 and 14.

Table 2 shows the values of titratable acidity. Titratable acidity ranged from 0.55 to 0.64 (% lactic acid) after 4 h of fermentation. No significant difference in titratable acidity was observed among the treatment group on days 1, 7 and 14.

Table 3 shows the syneresis of all yogurt samples. Syneresis of the probiotic and synbiotic yogurt samples (T2, T5 and T6) was not significantly different from that of the control yogurts on days 1, 7 and 14.

All yogurt samples appeared white upon instrumental color measurement. No significant difference in color was observed among the yogurt samples on days 1, 7 and 14 (Table 4). The storage had a minute effect on the color of the yogurts.

There was no significant difference in the studied sensory properties among yogurt samples (Table 5). The flavor, color, texture and mouth-feel values of all treatments were above 2.5, 2.8, 2.1 and 2.8, respectively, on a scale of 1-4.

Table 6 shows the Log CFU/mL of *S. thermophilus* and lactobacilli 1, 7 and 14 days after storage. The count 1 and 7 days after storage at 4°C were from 8.74 to 8.89, and from 8.52 to 8.82, respectively. No significant difference was observed in the *S. thermophilus* count among the studied yogurt samples on days 1 and 7. After 14 days of storage, the count was between 8.51 and 8.97 Log CFU/mL; T6 had a significantly higher count of *S. thermophilus* than the other samples.

Table 1: Mean±SEM pH of various yogurt samples during incubation period and storage up to 14 days

Samples[1]	Time							
	0 (h)	1 (h)	2 (h)	3 (h)	4 (h)	1 (Day)	7 (Day)	14 (Day)
T1	6.52±0.08	6.50±0.04	6.39±0.07	5.58±0.14	4.78±0.02	4.55±0.08	4.57±0.02	4.44±0.01
T2	6.51±0.07	6.47±0.07	6.08±0.04	5.11±0.06	4.78±0.10	4.62±0.08	4.55±0.11	4.49±0.03
T3	6.55±0.01	6.53±0.06	6.10±0.08	5.56±0.23	4.94±0.14	4.52±0.11	4.57±0.03	4.45±0.05
T4	6.58±0.04	6.47±0.07	5.94±0.12	5.13±0.06	4.73±0.10	4.60±0.02	4.51±0.07	4.44±0.03
T5	6.62±0.02	6.47±0.04	5.95±0.16	5.13±0.06	4.56±0.06	4.60±0.06	4.62±0.07	4.48±0.08
T6	6.61±0.03	6.40±0.07	5.87±0.19	5.04±0.02	4.55±0.08	4.50±0.03	4.52±0.07	4.44±0.04

[1]Abbreviations are: T1 = Yogurt, T2 = Probiotic yogurt, T3 = Yogurt + 1% inulin, 4 = Yogurt + 2% inulin, T5 = Synbiotic yogurt with 1% inulin, and T6 = Synbiotic yogurt with 2% inulin

Table 2: Mean±SEM titratable acidity values of various yogurt samples during incubation period and storage up to 14 days

Samples[1]	Titratable acidity (% Lactic acid)							
	0 (h)	1 (h)	2 (h)	3 (h)	4 (h)	1 (Day)	7 (Day)	14 (Day)
T1	0.16±0.01	0.17±0.01	0.20±0.01	0.34±0.02[a]	0.57±0.01	0.73±0.04	0.74±0.01	0.70±0.00
T2	0.16±0.01	0.17±0.01	0.25±0.01	0.44±0.03	0.57±0.03	0.68±0.02	0.76±0.03	0.73±0.01
T3	0.16±0.01	0.17±0.01	0.25±0.01	0.37±0.00	0.55±0.01	0.77±0.07	0.72±0.02	0.75±0.018
T4	0.16±0.01	0.17±0.01	0.26±0.02	0.41±0.01	0.55±0.04	0.70±0.00	0.76±0.02	0.72±0.01
T5	0.16±0.01	0.17±0.01	0.25±0.03	0.43±0.02	0.64±0.03	0.71±0.02	0.73±0.03	0.73±0.01
T6	0.16±0.01	0.18±0.00	0.27±0.04	0.46±0.02[b]	0.59±0.02	0.69±0.02	0.72±0.00	0.76±0.02

[1]Abbreviations are: T1 = Yogurt, T2 = Probiotic yogurt, T3 = Yogurt + 1% inulin, T4 = Yogurt + 2% inulin, T5 = Synbiotic yogurt with 1% inulin, and T6 = Synbiotic yogurt with 2% inulin. Different letters in the same columns indicate a statistically significant difference (P<0.05)

Table 3: Mean±SEM syneresis values of various yogurt samples during storage up to 14 days

Samples[1]	Syneresis (mL) on day		
	1	7	14
T1	12.41 ± 3.83	15.99 ± 0.77	17.46 ± 1.46
T2	14.66 ± 1.12	15.13 ± 1.56	17.79 ± 0.53
T3	11.78 ± 2.22	12.69 ± 1.91	15.31 ± 0.67
T4	9.58 ± 2.32	14.23 ± 1.07	14.85 ± 1.28
T5	12.58 ± 1.71	14.60 ± 1.28	17.33 ± 1.33
T6	11.55 ± 2.61	15.96 ± 0.40	16.36 ± 1.37

[1]Abbreviations are: T1 = Yogurt, T2 = Probiotic yogurt, T3 = Yogurt + 1% inulin, T4 = Yogurt + 2% inulin, T5 = Synbiotic yogurt with 1% inulin, and T6 = Synbiotic yogurt with 2% inulin

Table 4: Mean±SEM color values of various yogurt samples during storage up to 14 days

Samples[1]	Color on day								
	L*			a*			b*		
	1	7	14	1	7	14	1	7	14
T1	66.4±0.4	66.3±0.2	64.9±0.3	4.5±0.2	4.9±0.2	5.4±0.1	11.8±0.3	11.9±0.7	13.4±0.4
T2	66.1±0.3	65.9±0.4	64.4±0.6	5.0±0.0	4.4±0.1	5.1±0.1	12.9±0.3	11.8±0.3	13.4±0.2
T3	66.0±0.3	66.6±0.2	64.7±0.6	4.6±0.1	4.5±0.2	5.3±0.2	12.2±0.2	11.9±0.8	13.5±0.5
T4	65.2±0.3	66.2±0.3	64.8±0.8	5.0±0.2	4.4±0.1	5.2±0.1	12.4±0.4	11.7±0.4	12.5±0.3
T5	64.6±0.9	65.5±0.2	65.6±0.5	4.7±0.1	4.4±0.1	5.1±0.1	11.6±0.2	11.3±0.3	13.0±0.3
T6	65.9±0.4	65.7±0.2	65.8±0.5	4.9±0.1	4.6±0.1	5.0±0.2	13.1±0.3	12.1±0.2	13.1±0.5

[1]Abbreviations are: T1 = Yogurt, T2 = Probiotic yogurt, T3 = Yogurt + 1% inulin, T4 = Yogurt + 2% inulin, T5 = Synbiotic yogurt with 1% inulin, and T6 = Synbiotic yogurt with 2% inulin

Table 5: Mean±SEM sensory scores of various yogurt samples during storage up to 14 days, n =30

Samples[1]	Sensory scores on day											
	Flavor			Color			Texture			Mouth-feel		
	1	7	14	1	7	14	1	7	14	1	7	14
T1	2.6 ±0.2	2.7 ±0.2	2.9 ±0.1	3.1 ±0.2	2.8 ±0.2	3.1 ±0.1	2.3 ±0.2	2.1 ±0.2	2.6 ±0.2	2.4 ±0.2	2.6 ±0.2	3.2 ±0.1
T2	2.8 ±0.2	3.1 ±0.2	3.0 ±0.2	3.1 ±0.2	2.9 ±0.2	3.2 ±0.1	3.1 ±0.2	2.6 ±0.2	3.0 ±0.1	3.1 ±0.2	3.0 ±0.2	3.2 ±0.1
T3	2.8 ±0.3	2.8 ±0.2	2.8 ±0.2	3.1 ±0.2	2.9 ±0.2	3.1 ±0.1	2.8 ±0.2	2.2 ±0.2	2.8 ±0.2	2.9 ±0.2	2.7 ±0.2	3.1 ±0.2
T4	3.0 ±0.2	2.9 ±0.2	3.1 ±0.1	2.8 ±0.3	3.0 ±0.2	3.3 ±0.1	2.7 ±0.3	2.6 ±0.2	2.6 ±0.2	3.0 ±0.2	3.1 ±0.1	3.4 ±0.1
T5	2.5 ±0.3	2.9 ±0.1	3.1 ±0.2	2.9 ±0.3	3.0 ±0.2	3.2 ±0.1	2.3 ±0.2	2.6 ±0.2	2.9 ±0.1	3.2 ±0.2	3.0 ±0.1	3.1 ±0.2
T6	2.8 ±0.1	2.9 ±0.2	3.2 ±0.1	3.0 ±0.2	3.0 ±0.2	3.2 ±0.1	2.7 ±0.2	2.5 ±0.2	3.1 ±0.1	3.0 ±0.2	2.9 ±0.1	3.6 ±0.1

[1]Abbreviations are: T1 = Yogurt, T2 = Probiotic yogurt, T3 = Yogurt + 1% inulin, T4 = Yogurt + 2% inulin, T5 = Synbiotic yogurt with 1% inulin, and T6 = Synbiotic yogurt with 2% inulin

The count of *L. delbrueckii* ssp. *bulgaricus* after 1 and 7 days of storage at 4°C was from 7.25 to 7.54, and from 6.57 to 7.20 Log CFU/mL, respectively. After 14

Table 6: Mean±SEM bacterial count of the various yogurt samples during storage up to 14 days

Samples[1]	Bacterial counts (Log CFU/mL) on day		
	1	7	14
S. thermophilus			
T1	8.80 ± 0.08	8.59 ± 0.10	8.95 ± 0.06
T2	8.82 ± 0.05	8.52 ± 0.04	8.70 ± 0.04
T3	8.74 ± 0.11	8.66 ± 0.11	8.51 ± 0.02
T4	8.84 ± 0.19	8.72 ± 0.10	8.63 ± 0.05
T5	8.82 ± 0.05	8.62 ± 0.01	8.79 ± 0.01
T6	8.89 ± 0.05	8.82 ± 0.05	8.97 ± 0.10[bc]
L. delbrueckii ssp. *bulgaricus*			
T1	7.54 ± 0.16	6.57 ± 0.15	5.30 ± 0.19[a]
T2	7.40 ± 0.02	6.65 ± 0.43	6.55 ± 0.5
T3	7.25 ± 0.17	6.95 ± 0.11	5.62 ± 0.02[a]
T4	7.36 ± 0.11	6.58 ± 0.04	6.11 ± 0.36
T5	7.41 ± 0.05	6.89 ± 0.21	6.78 ± 0.17
T6	7.43 ± 0.01	7.20 ± 0.19	7.19 ± 0.19[b]
L. acidophilus			
T1	-	-	-
T2	7.41 ± 0.01	7.35 ± 0.05	6.71 ± 0.40
T3	-	-	-
T4	-	-	-
T5	7.36 ± 0.03	7.19 ± 0.05	7.1 ± 0.12
T6	7.35 ± 0.03	7.30 ± 0.13	7.12 ± 0.16

[1]Abbreviations are: T1 = Yogurt, T2 = Probiotic yogurt, T3 = Yogurt + 1% inulin, T4 = Yogurt + 2% inulin, T5 = Synbiotic yogurt with 1% inulin, and T6 = Synbiotic yogurt with 2% inulin. Different letters in the same column indicate a statistically significant difference (P<0.05)

day of storage, the mean count of *L. delbrueckii* ssp. *bulgaricus* in the T6 sample was significantly higher than in the T1 and T3 samples. On day 1, the count of *L. acidophilus* exceeded 7.35 Log CFU/mL. The count was not significantly different between the probiotic and synbiotic yogurt samples. After 14 days of storage, the count of *L. acidophilus* decreased in the probiotic and synbiotic yogurt samples, but remained more than 6.71 Log CFU/mL.

Discussion

Several studies have reported the effect of inulin on the physico-chemical properties of dairy products. We found that the addition of 1% and 2% inulin to yogurt did not affect its pH and acidity during storage. These findings are in keeping with those of Guven *et al.* (2005), who also reported that the use of inulin did not significantly affect the pH of yogurt. We also showed that the addition of inulin plus *L. acidophilus* to yogurt has no affect on the rate of increase in pH and acidity compared to adding either inulin or *L. acidophilus* alone after 4 h of fermentation and during the storage up to 14 days. The results revealed that syneresis increased in all samples with storage. However, there was a tendency toward a lower syneresis after 14 days of storage of the yogurt by adding more inulin. These findings were in keeping with reports that yogurt containing long chain inulin (HP) had lower syneresis than a control yogurt (Aryana *et al.*, 2007). A possible explanation for these observations would be that inulin has a high water holding capacity (Douglas, 2005). There was no significant difference in the sensory (flavor, color, texture, mouth-feel) scores of the yogurt samples during the storage and between treatments. Aryana and McGrew (2007) reported that yogurt with 1.5% inulin HP had better body and texture compared to a control yogurt. Inulin levels (1% and 2%) had no significant effects on the count of *S. thermophilus* and lactobacilli spp. in synbiotic yogurt on days 1 and 7. Higher counts of *L. acidophilus* were observed in yogurts containing inulin compared to probiotic yogurt, but the difference was not statistically significant. Aryana and McGrew (2007) reported a decreased count of *L. acidophilus* in yogurts with and without inulin of various chain lengths over 28 days of storage at 4°C. We also found that the count of *L. acidophilus* declined with time in probiotic and synbiotic yogurt. However, the final count of *L. acidophilus* was above the "therapeutic minimum" of 1.0×10^6 CFU/mL (Sellars, 1991). The count of *L. delbrueckii* ssp. *bulgaricus* declined with time in all treatment groups, although the synbiotic yogurt containing 2% inulin had a higher *L. delbrueckii* ssp. *bulgaricus* count. In addition, the *L. delbrueckii* ssp. *bulgaricus* count was significantly higher with the increase in inulin level. In conclusion, this study showed that inulin can enhance the nutritional benefits of low fat synbiotic yogurt without affecting its physico-chemical properties. The addition of inulin to milk increases the viability of *L. acidophilus*, *S. thermophilus* and *L. delbrueckii* ssp. *bulgaricus* during the storage of synbiotic yogurt.

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