ORIGINAL RESEARCH

An investigation of some properties of banana yogurts made with commercial ABT-2 starter culture during storage

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*Five yogurt batches were prepared with banana puree and sugar combinations and ABT-2 (*Streptococcus salivarius *ssp.* thermophilus, Lactobaccilus acidophilus *and* Bifidobacterium *spp.) as starter culture. The addition of fruit influenced (*$P < 0.05$*) the syneresis, titratable acidity and pH, whereas the viscosity value, and* S. salivarius *ssp.* thermophilus, L. acidophilus *and* Bifidobacterium *spp. counts were found to be insignificant. Storage time significantly (*$P < 0.05$*) affected the syneresis, titratable acidity, pH value, and* S. salivarius *ssp.* thermophilus *counts, while the viscosity value and* L. acidophilus *and* Bifidobacterium *spp. counts were not affected. The sensory evaluations showed that the sample B was most preferred by panellists.*

Keywords ABT starter culture, Banana puree, Probiotics, Storage, Yogurt.

INTRODUCTION

Probiotics are usually defined as live microbial food supplements that exert beneficial effects for the host by improving its intestinal microbial balance (Ziemer and Gibson 1998; Saarela *et al*. 2000; Lourens-Hattingh and Viljoen 2001; Isolauri *et al*. 2004; Bergamini *et al*. 2005). Also, fermented milks containing probiotic micro-organisms are generally categorized as functional foods, and along with prebiotics represent the largest segment of the functional foods market worldwide, especially Europe, Japan and Australia (Lucas *et al*. 2004; Ross *et al*. 2005).

Some health benefits of probiotic foods have been attributed to fermented dairy products, such as reduction in serum cholesterol, alleviation of lactose intolerance, reduction of diarrhoea, prevention and suppression of colon cancer and stimulation of the immune system (Gilliland *et al*. 1985; Marteau *et al*. 1993; Sanders 1994; Perdigon *et al*. 1995; Dubey and Mistry 1996; Salminen *et al*. 1998; Kasper 1998; Ouwenhand and Salminen 1998; Saarela *et al*. 2000; Lourens-Hattingh and Viljoen 2001; Saarela *et al*. 2002; Itsaranuwat *et al*. 2003; Donkor *et al*. 2006). The strains with beneficial properties mostly belong to the genera *Bifidobacterium* and *Lactobacillus*; and the products made with these bacteria are classified as bio-yogurt, containing *Lactobacillus acidophilus* and *Bifidobacterium bifidum* (AB-yogurt) or ABT (*L. acidophilus*, bifidobacteria, *Streptococcus salivarius* ssp. *thermophilus*) starter culture (Lourens-Hatting and Viljoen 2001; Isolari *et al*. 2004). However, to achieve an optimal potential therapeutic effect, the number of these organisms in a probiotic product should meet a suggested minimum of $> 10^6$ colony-forming units per millilitre (cfu/mL) (Kurmann and Rasic 1991). According to Tamime *et al*. (1995), regular consumption of 400–500 g/week of AB-yogurt, containing 10^6 viable cells per mL, would provide these numbers.

The composition of product, the presence of preservatives as a result of added fruits and nuts, and the availability of growth factors are also reported to affect the growth and viability of yogurt and probiotic bacteria (Dave and Shah 1998). Inulin and fructo-oligosaccharides are probably the most commonly used prebiotics, and are nondigestible carbohydrates used increasingly in food preparations for human consumption (Rao 2001; Holzapfel and Schillinger 2002). Fructo-oligosaccharides are widely found in plants and vegetables, including banana, plum, onion, shallot, chicory and artichoke (L'homme *et al*. 2003). The aim of this study was to investigate the changes in some physicochemical, microbiological and sensory properties in banana yogurts made with a commercial probiotic culture (ABT-2) during storage.

MATERIALS AND METHODS

Materials

Raw cow's milk used in the manufacture of yogurt was obtained from the dairy farm of Atatürk University. Skim milk powder (SMP), starch and banana were purchased from local shops in Erzurum. A commercial probiotic starter culture (ABT-2)

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was used (Peyma-Chr. Hansen, Istanbul, Turkey) containing *S. salivarius* ssp. *thermophilus*, *L. acidophilus* and *Bifidobacterium* species.

Preparation of banana puree

The fruit skin was removed manually and the peeled fruit was then pureed. The puree was then pasteurized at 90 ± 1°C for 5 min and transferred to sterile glass jars, which after cooling were kept at ambient temperature until used in the experimental yogurts.

Manufacture of experimental yogurts

SMP (30 g/L) was added to the yogurt milk after clarification and the milk was heated to 90°C for 10 min, cooled to 38 ± 1°C and divided into five equal parts (5-L sterile glass cups) coded with letters from A to E. The milks were then inoculated with ABT-2 starter culture at the rate of 0.03% (w/w). Inoculated milks were incubated at 37 ± 1°C until the pH reached 4.6 (approximately 10 h), and were then stored for 24 h at 4°C. One batch of yogurt was then taken as control (A), and the remaining batches were prepared with one of the mixtures of B [10% (w/w) banana puree and 5% (w/w) sugar], C [10% (w/w) banana puree and 10% (w/w) sugar], D [15% (w/w) banana puree and 5% (w/w) sugar] and E [15% (w/w) banana puree and 10% (w/w) sugar], respectively. All experimental yogurts were divided into parts of approximately 200 g and stored at 4 ± 1°C for 14 days. The analyses were carried out after 1, 7 and 14 days of storage. Two trials were carried out, and the results are the means of these trials.

Analytical procedures

Total solids and ash contents of milk, SMP and experimental yogurts were determined by the gravimetric method, fat content by the Gerber method, and protein by the Kjeldahl method as described by Kurt *et al*. (1996). Solids nonfat contents of yogurt samples were determined by calculation (Table 1). The apparent viscosities of yogurts were measured using an RV 8-model viscometer (Selfe Elee Ltd, Wickford, Essex, UK). The spindle used was 4 cm in diameter and the speed was kept constant at 20 r.p.m., and the temperature was 4 ± 1°C. Data were directly read from instrument as centipoises (cP) (Abrahamsen and Holman 1980). Syneresis was estimated using a drainage test according to Atamer and Sezgin (1986): a 25-g yogurt sample was weighed and filtered at 4 ± 1°C and after 2 h of drainage, the volume of filtrate collected in a graduated cylinder was measured and used as an index of syneresis. The pH was measured using a pH meter (model WTW pH-340-A, Weilheim, Germany) fitted with a combined glass electrode. The titratable acidity

Table 1 The mean values of some properties of raw cow's milk and skim milk powder (SMP) ($n = 3$)

	Total solids (%)	*Fat (%)*	*Solids non-fat (SNF) (%)*	*Protein (%)*	*Ash (%)*	*Titratable acidity (lactic acid %)*	*pH*
Cow's milk	11.75 ± 0.578	3.10 ± 0.000	8.65 ± 0.000	3.66 ± 0.920	0.73 ± 0.097	0.17 ± 0.029	6.51 ± 0.163
SMP	96.06 ± 0.009	1.00 ± 0.000	—	36.00 ± 0.000	8.24 ± 0.012	0.14 ± 0.027	6.71 ± 0.382

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was determined as lactic acid percentage by titrating with 0.1 N NaOH, using phenolphthalein as an indicator (Kurt *et al*. 1996). The counts of *Streptococcus salivarius* ssp. *thermophilus* were enumerated on M17 agar (Oxoid Ltd, Basingstoke, Hampshire, UK) by incubating the plates aerobically at 37°C for 24 ± 1 h (Rybka and Kailasapathy 1996). MRS agar (Oxoid Ltd) was used for enumeration of the total viable counts of *L. acidophilus* and *Bifidobacterium* species (Rybka and Kailasapathy 1996; Vinderola *et al*. 1999, 2000). *L. acidophilus* counts were obtained under aerobic conditions at 37°C for 72 ± 1 h and *Bifidobacterium* spp. cultures were anaerobically (Anerocult A system; Merck, Darmstadt, Germany) grown at 37°C for 72 ± 1 h (Vinderola *et al*. 2000; Gueimonde *et al*. 2004). The results were expressed as cfu/g. A group of six panellists evaluated the experimental yogurts by grading them on a scale of 1–5 (unacceptable/excellent) on days 1, 7 and 14 of storage (Bodyfelt *et al*. 1988; Turkish Standard Institution 1989). Statistical analysis was performed according to a randomized complete block design by a 3 (fruit) × 2 (sugar) × 3 (storage period) factorial experiment. The parameters of experimental yogurts were estimated by generalized linear model ANOVA by using Minitab® statistical software (MINITAB 2005). Different groups were analysed by Duncan's Multiple Range Test.

RESULT AND DISCUSSION

Overall composition

The general compositions of cow's milk and SMP used for the yogurt production and the experimental yogurts are shown in Tables 1 and 2, respectively.

The compositional values of fresh milk and SMP used in yogurt production were in agreement with TS-1018, the standard of raw cow's milk, and TS-1329, the standard of milk powder (Turkish Standard Institution 2002, 2005).

General composition of the experimental yogurts

Total solids, fat, solids nonfat, protein and ash contents of the yogurts were analysed on day 1 of storage in both experiments, and the mean values obtained are presented in Table 2. When considering the content of total solids of milk used in the yogurt production, the amount of SMP added and the proportions of fruit-sugar combinations, it can be concluded that these results were as expected.

Physicochemical characteristics of the experimental yogurts

Apparent viscosity, syneresis, titratable acidity and pH values of the produced yogurts, after storage at 4°C for 1, 7 and 14 days, are shown in Table 3.

Apparent viscosity

Although a slightly variation was observed among the samples, there were no statistically significant ($P > 0.05$) differences between the control yogurt and the other experiments made with banana puree and sugar in different ratios. The lowest apparent viscosity was observed on day 1 of storage, and the viscosity values increased with period of storage, but this was not statistically significant ($P > 0.05$). This increase could be attributed to the conjunction of the agglutination of fat globules and some proteins exposed to aggregation in low temperatures (Walstra and Jenness 1984; Bienvenue *et al*. 2003).

Syneresis

Syneresis is generally defined as spontaneous water release from the gel caused by gel contraction (Walstra and Jenness 1984; Pears and Mackinlay 1989). The highest mean value (5.56 mL/25 g) of syneresis was found in sample A (control) and the lowest mean value (3.99 mL/25 g) in sample E. As seen in Table 3, the addition of fruit-sugar caused a decrease of syneresis values in all samples of fruit yogurt, and differences between the control and these samples were significant ($P < 0.05$)

Table 2 The mean values of some chemical properties obtained from the experimental yogurts ($n = 2$)

Experimental yogurts	*Total solids (%)*	*Fat (%)*	*Solids nonfat (SNF) (%)*	*Protein (%)*	*Ash (%)*
A (control) (with no banana puree and sugar)	13.94 ± 0.544	3.6 ± 0.000	10.34 ± 0.544	4.27 ± 0.332	0.83 ± 0.071
B [10% (w/w) banana puree and 5% (w/w) sugar]	16.79 ± 0.297	2.7 ± 0.424	14.09 ± 0.127	3.94 ± 0.389	0.84 ± 0.141
C [10% (w/w) banana puree and 10% (w/w) sugar]	21.06 ± 0.431	3.0 ± 0.000	18.06 ± 0.431	3.72 ± 0.583	0.73 ± 0.075
D [15% (w/w) banana puree and 5% (w/w) sugar]	17.73 ± 0.115	2.9 ± 0.141	14.83 ± 0.027	4.35 ± 1.059	0.89 ± 0.033
E [15% (w/w) banana puree and 10% (w/w) sugar]	21.58 ± 0.024	2.6 ± 0.566	18.98 ± 0.590	4.24 ± 0.543	0.62 ± 0.077

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Table 3 Mean values of some physicochemical properties of the experimental yogurts and their statistical evaluations in terms of fruit-sugar ratios and storage period (days)[a]

Experimental yogurts	*Apparent viscosity (cP)*[b]	*Syneresis (mL/25 g)*	*Titratable acidity (lactic acid %)*	*pH*
A (control) (with no banana puree and sugar)	2876.7	5.56a	1.07a	4.34c
B [10% (w/w) banana puree and 5% (w/w) sugar]	2648.3	4.46b	0.95b	4.40a
C [10% (w/w) banana puree and 10% (w/w) sugar]	2933.3	4.49b	0.92bc	4.37b
D [15% (w/w) banana puree and 5% (w/w) sugar]	2943.3	4.28b	0.94bc	4.37b
E [15% (w/w) banana puree and 10% (w/w) sugar]	2826.7	3.99c	0.90c	4.36b
Storage time (days)	2701.0	4.65a	0.93b	4.38a
1	2701.0	4.65a	0.93b	4.38a
7	2706.0	4.34b	0.96ab	4.37ab
14	3130.0	4.46ab	0.98a	4.35b

[a]Different letters indicate significant differences ($P < 0.05$) between experiments and days of storage.
[b]Centipoise.

statistically. This could be explained by the availability of fibre from banana, which has high water-holding capacity (Garcia-Perez *et al*. 2005). In general, storage time significantly ($P < 0.05$) affected syneresis. The mean value of whey separation obtained after 1 day of storage was significantly higher than those of yogurts examined after 7 and 14 days of storage (Table 3). Martin-Diana *et al*. (2003) obtained similar results in set-type fermented milks supplemented with whey protein concentrate (WPC), suggesting that the addition of WPC reduced whey separation by improving water-holding capacity. A similar result was also found by Guler-Akin (2007).

Titratable acidity

For balanced flavour development in yogurt, titratable acidity values should be within certain limits (Tamime and Deeth 1980; Sezgin 1981). There were significant differences ($P < 0.05$) in titratable acidity between the control yogurt and the yogurts made with fruit. In the range 0.90–1.07%, sample A (control yogurt, 0% fruit-sugar) had the highest mean value, and the sample E (with 15% of banana puree and 10% sugar) had the lowest mean value of titratable acidity (Table 3). This could be attributed to the osmotic effect from the added sugar (Tamime and Robinson 1999). The lowest mean value of titratable acidity was found on day 1 of storage, but the highest value was found on day 14 of storage, and these differences were statistically ($P < 0.05$) significant (Table 3). Some authors reported similar results (Bonczar *et al*. 2002; Katsiari *et al*. 2002). This might be due to the acid production in the experimental yogurts during storage as a result of the fermentation of lactose by the action of the starter cultures (Rasic and Kurmann 1978).

pH

As seen in Table 3, the pH values of the fruit yogurt samples were higher than those of the control (sample A). This was probably due to the availability of fibre from banana added to the yogurts (Aportela-Palacious *et al*. 2005). The differences between the control and the fruit yogurt samples were found to be statistically ($P < 0.05$) significant. In general, the pH values of all samples decreased during storage, and these differences were found to be significant ($P < 0.05$) statistically. This can be explained by further metabolic activity of starter cultures during storage (Bonczar *et al*. 2002).

Microbiological characteristics of the experimental yogurts

S. salivarius ssp. *thermophilus* is the main organism responsible for fermentation in ABT cultures (Shah 2000). The changes in the mean viable counts of *S. salivarius* ssp. *thermophilus* in the experimental yogurts and in the storage times are given in Table 4. There were no significant ($P > 0.05$) differences between the control yogurt and the fruit yogurts in terms of the viable cell counts. Çon *et al*. (1997) reported that the addition of fruit flavours such as cherries, oranges, strawberries and bananas, or sweeteners to yogurt did not show a significant effect on total bacteria. Also, Vinderola *et al*. (2002) suggested that there are insufficient data regarding the effect that fruits may have on the survival of microflora of these products. As is evident from Table 4, the counts of *S. salivarius* ssp. *thermophilus* showed a slow decline

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Table 4 Mean values of viable bacteria counts of the experimental yogurts and their statistical evaluations in terms of fruit-sugar ratios and storage time (days)[a]

Experimental yogurts	S. salivarius *ssp.* thermophilus *(log cfu/g)*	L. acidophilus *and* Bifidobacterium *spp. (log cfu/g)*
A (control) (with no banana puree and sugar)	6.66	8.01
B [10% (w/w) banana puree and 5% (w/w) sugar]	6.67	8.01
C [10% (w/w) banana puree and 10% (w/w) sugar]	6.77	8.11
D [15% (w/w) banana puree and 5% (w/w) sugar]	6.34	8.11
E [15% (w/w) banana puree and 10% (w/w) sugar]	6.75	8.35
Storage time (days)	7.24a	8.43
1	7.24a	8.43
7	6.80ab	8.01
14	5.87b	7.91

[a]Different letters indicate significant differences ($P < 0.05$) between experiments and days of storage.

Table 5 Mean values of sensory characteristics of the experimental yogurts and their statistical evaluations in terms of fruit-sugar ratios and storage time (days)[a]

Experimental Yogurts	*Appearance*	*Consistency (by spoon)*	*Consistency (by mouth)*	*Smell*	*Taste intensity*	*Overall acceptability*
A (control) (with no banana puree and sugar)	4.13a	3.52	3.82	4.06	4.03a	19.56a
B [10% (w/w) banana puree and 5% (w/w) sugar]	3.94a	3.84	3.97	3.99	4.00a	19.74a
C [10% (w/w) banana puree and 10% (w/w) sugar]	3.57ab	3.81	3.99	4.04	3.77ab	19.18ab
D [15% (w/w) banana puree and 5% (w/w) sugar]	3.17b	3.46	3.54	3.20	3.20b	17.06b
E [15% (w/w) banana puree and 10% (w/w) sugar]	3.66ab	3.78	3.69	4.05	3.44ab	18.62ab
Storage time (days)	3.89	3.70	3.95	3.99	3.77	19.30
1	3.89	3.70	3.95	3.99	3.77	19.30
7	3.58	3.82	3.85	4.04	3.63	18.92
14	3.61	3.53	3.61	3.88	3.66	18.28

[a]Different letters indicate significant differences ($P < 0.05$) between experiments and days of storage.

(0.44 log cycle) during storage up to day 7. Later the decrease was very low (0.93 log cycle) between day 7 and 14 of storage, and the differences between the days of storage were significant ($P < 0.05$). Similar results were also reported by Dave and Shah 1998).

The changes of viable counts of *L. acidophilus* and *Bifidobacterium* spp. in the experimental yogurts during manufacture and storage period are shown in Table 4. As seen, the highest mean count (8.35 log cfu/g) was found in sample E, and the lowest mean count (8.01 log cfu/g) was found in sample A (control) and sample B. It was observed that a higher addition of fruit and sugar leads to an increase in the viable counts of these bacteria, but the differences between the yogurt batches, including control group were not significant ($P > 0.05$) (Table 4). On the other hand, total viable counts of *L. acidophilus* and *Bifidobacterium* spp. decreased slightly during the storage, but remained at sufficient levels (> 6 log cfu/g) for up to 14 days. According to the variance analysis, there were no statistically significant ($P > 0.05$) differences in the viable counts of *L. acidophilus* and *Bifidobacterium* spp. between days of the storage.

Sensory evaluations of the experimental yogurts

The mean scores for the sensory characteristics of the experimental yogurts are presented in Table 5. The addition of fruit to yogurt in different proportions significantly affected ($P < 0.05$) the scores for appearance, taste intensity and overall acceptability, whereas a significant effect ($P > 0.05$) was not observed for consistency (by spoon and by mouth) or aroma by the addition of fruit. It was also

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observed that the storage time did not affect the evaluations of panellists in all sensory parameters tested (Table 5).

CONCLUSIONS

All batches of yogurt made with banana and a commercial probiotic starter culture (ABT-2) revealed different patterns in manufacture and storage time. Addition of fruit and sugar at different levels did not have any marked effect on the apparent viscosity, viable counts of *S. salivarius* ssp. *thermophilus, L. acidophilus* and bifidobacteria, and scores of consistency (by spoon and mouth) or aroma. The values of syneresis, titratable acidity and pH, as well as the scores for appearance and overall acceptability were significantly affected by the added fruit and sugars. On the other hand, the values of apparent viscosity, the counts of *L. acidophilus* and bifidobacteria, and all sensory evaluations performed by panellists were not significantly affected by storage time, except for the counts of *S. salivarius* ssp. *thermophilus*. In the sensory evaluations, sample B, containing 10% banana puree and 5% sugar, was preferred more than sample A (control) by panellists. It was concluded that a natural fruit such as banana can be used in the manufacture of bio-yogurt made with a commercial probiotic starter culture (i.e. ABT-2), but further investigations are needed to achieve the best physicochemical and sensory characteristics of the final product, and to optimize growth and cell viability of these starter cultures during production and storage.

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