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Physicochemical, Microbiological and Sensory Characteristics of Using Different Probiotic Fermented Milk

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Abstract: The objectives of this study were to determine how fermented milk could be produced using different combinations of probiotic cultures and also to determine their physical, chemical, microbiological and organoleptic properties during storage. The results indicate that using probiotics with the standard yoghurt bacteria as adjunct culture had positive effects on the physical, chemical and microbiological properties of the products. Moreover the majority of the panelists in the sensory evaluation described the probiotic fermented milk samples as having better aroma/flavour. Furthermore, it was seen that acidity and durability of aroma/flavour until the end of the storage period was better than normal yoghurt drink.

Key words: Functional food, probiotic, fermented milk

INTRODUCTION

It is seen that there was an increasing interest in the opportunities of new foods with health promoting properties and nutritional values. Today, these functional products targeting gut health have attracted great attention and a wide range of probiotic and prebiotic containing ones are now available. Probiotic microorganisms belong mainly to the genus *Bifidobacterium* and *Lactobacillus*, also *Enterococcus*, *Pediococcus* or *Saccharomyces* strains have also been considered too (Sanchez *et al*., 2008).

Their associated health benefits include treatment of diarrhea, relief of lactose intolerance, enhancement of the immune system, prevention of infections, possible delay or prevention of colon cancer and reduction of serum cholesterol (Leahy *et al*., 2005; Parvez *et al*., 2006; Sanchez *et al*., 2008). Viability of probiotic bacteria to high counts (at least 10^7 cfu/g or mL^{-1} of product) is recognized as an important requirement during manufacturing and marketing of probiotic foods in order to achieve the claimed health benefits (Martin-Diana *et al*., 2003). Traditionally, probiotic microorganisms have been included in dairy products, fruit juices or meat products. However in terms of the growth and viability of probiotic bacteria in retail products, fermented milks are excellent vehicles for the transfer of selected strains to humans (Itsaranuwat *et al*., 2003).

Currently, the slow pace of regulatory approvals for functional foods and bioactive ingredients is constantly being challenged by the rapid increase in innovative ingredients and a desire to make health claims. Although Japan leads in the development and approval of functional foods, the USA and Europe are also closely behind. This has allowed these countries to lead in the innovation and capture of intellectual property in the area of functional foods and bioactive ingredients. Today, supermarket shelves in US, Europe and Japan are full of functional dairy beverages with probiotics, prebiotics, omega 3, sterols and many other components (Sharma, 2005).

The objective of this work is to obtain fermented milks with probiotics that has a satisfactory quality for consumers and also to determine the physical, chemical and sensory properties of these new fermented milks.

MATERIALS AND METHODS

Raw cow milk used in the production of fermented milks was obtained from Pýnar Sut Co. (Izmir, Turkey) within 1 h of milking and kept at 4°C during transportation to the laboratory. The MYE 96-98 cultures containing *Streptococcus thermophilus* and *Lactobacillus bulgaricus* obtained from Maysa Gıda Co. and TFM 001 cultures containing *Str. thermophilus* and *Lactobacillus lactis* obtained from Ezal® /Texel, France, were used as starter cultures in probiotic fermented milk production. The commercial culture containing *Str. thermophilus*, *Lb. bulgaricus*, *Lb. acidophilus and Bifidobacterium* spp. (Ezal® /Texel, France) and L.B.A culture containing *Lb. casei* subsp. *rhamnosus* (Ezal® /Texel, France) were obtained from Maysa Gıda Co. and also, A/S10-12 culture containing *Lb. acidophilus* was also obtained from Chr. Hansen (Chr. Hansen, Hoersholm-Denmark).

Production of fermented milk: Milk used in the production of probiotic fermented milk was pasteurized at 90°C for 15 minutes. The pasteurization norm is appropriate for development of products that stimulated development of probiotics. Pasteurized milk was divided in four equal parts. A part of milk was used as the control

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product that only contains the yoghurt culture containing *Lb. bulgaricus* and *Str. thermophilus*. In the other three parts, three different probiotic culture mixtures were used to produce probiotic fermented milk products. So, four different experimental fermented milk samples were produced. Culture contents and rates of these four products are as follows:

- Yoghurt culture (control) (2.5-3%) (FD1)
- *Str. thermophilus/Lb. bulgaricus/Lb. acidophilus/ Bifidobacterium* spp. (3.5-4.0%) (FD2)
- *Str. thermophilus/Lb. lactis* (0.5-1%)+*Lb. acidophilus* (1.5-2%) (FD3)
- *Lb. casei* subsp. *rhamnosus* (0.75%) + Yoghurt culture (2.5-3%) (FD4)

The incubation for FD2 and FD3 was held for 4-5 hours at 37°C and for FD1 and FD4 at 42°C for 2.5-3 hours, until the pH of milk decrease to 4.5-4.6. During pre-cooling at 20°C in water bath, the fermented milk samples were stirred and filled into 200 mL of glass bottles and tapped with reclosable taps in aseptic conditions. The fermented milk samples were stored at 4°C for 10 days. On the 1st, 5th and 10th days of storage some chemical, physical, microbiological and sensory properties were determined.

Chemical and physical analyses: The fermented milk samples were analyzed for total solids, fat, lactose, protein, titratable acidity (%), pH (Oysun, 1996), acetaldehyde (Robinson *et al*., 1977) and tyrosine content (Hull, 1947).

Microbiological analyses: *Str. thermophilus* and *Lb. bulgaricus* counts and probiotic bacteria counts were enumerated in each fermented milk samples. Each sample was serially diluted to 10^{-6} (taking 1 ml into the 9 ml of media) with Ringers' solution. Appropriate dilutions were prepared using the following media:

- MRS agar (Merck, Darmstadt/Germany) for the enumeration of *Lb. bulgaricus*; was incubated anaerobically at 42±2°C for 3 days (Dave and Shah, 1997)
- M17 agar (Merck, Darmstadt/Germany) for the enumeration of *Str. thermophilus*; was anaerobically incubated at 37±2°C for 3 days (Bracquart, 1981)
- NPNL-MRS agar: MRS agar containing nalidiccic acid, paromycine sulphate and lithium chloride for the enumeration of *Bifidobacterium* spp. (Dave and Shah, 1997); was incubated at 37°C for 72 hours anaerobically
- MRS agar containing D-sorbitol (Sartorius AG, Goettingen/Germany) for the enumeration of *Lb. acidophilus*; was incubated at 37°C for 72 hours anaerobically (Dave and Shah, 1997)
- LAMVAB MRS agar containing vancomycine and bromocresol green for the enumeration of *Lb. casei* subsp. *rhamnosus* was anaerobically incubated at 37°C for 72 hours
- MRS agar for the enumeration of *Lb. lactis*; was incubated anaerobically at 42°C for 3 days (Dave and Shah, 1997)

Sensory analysis: Samples were evaluated for their sensory properties (aroma/flavour, consistency and overall acceptability) on a 1-10 point hedonic scale performed by a panel of six judges experienced with fermented dairy products (Bodyfelt *et al*., 1988).

RESULTS AND DISCUSSION

Chemical and physical properties: Some properties of fermented milk samples and changes in these properties during the storage period were given in Table 1. It was seen that total solid values of the fermented milks had been between 8.58 and 8.63%. Muir *et al.* (1999) studied on various fermented milks like buttermilk, kefir and yoghurt drinks. They had found total solid contents between 7.99-13.33. These values were similar to results obtained from this study.

The total solids content varied in small quantities over the storage period of the samples made with different cultures. After first day of storage, fat contents of fermented milks produced with different culture types was nearly 1.45%. These findings were consistent with reports by other researchers (Muir *et al*., 1999; Huerta-Gonzalez and Wilbey, 2001; Irigoyen *et al*., 2005). Lactose levels changed significantly according to the initial lactose levels of milk during the first 24 h of storage. These levels practically remained constant during the storage period. The results obtained in our study were consistent with those reported by Katsiari *et al*. (2002), in which larger decrease in lactose content brought about by the bacteria in the culture and then remained constant during storage period. Mean values of protein content was 2.34 and 2.98% during the first day of storage. Due to the fact that low total solid content, protein contents of the products were lower than normal. Then, protein levels practically remained constant over the storage period. The data are inconsistent with findings of Muir *et al.* (1999) who found higher protein contents in fermented milk drinks.

Titratable acidity increased in all samples during the fermentation and the main increase was observed in the first day of storage. The samples containing probiotic bacteria had lower acidity than control samples. The decrease attitude of pH values in fermented milk samples were found to be slower when compared to the values observed in the control group (FD1). Ostlie *et al.* (2003), reported a reduction in pH from 6.7 to 3.9 and 4.4 after 24 h of incubation, in their study that was about the

Table 1: Physical and chemical properties of fermented milk samples (n = 2)

		Samples			
	Days	FD1	FD2	FD3	FD4
Total solids (%)	1	8.59±0.05	8.63±0.08	8.58±0.04	8.59±0.16
	5	8.47±0.00	8.69±0.09	8.67±0.11	8.66±0.16
	10	8.59±0.11	8.70±0.09	8.61±0.14	8.72±0.05
Fat (%)	1	1.45±0.00	1.42±0.03	1.45±0.00	1.50±0.00
	5	1.42±0.35	1.40±0.07	1.42±0.03	1.45±0.00
	10	1.45±0.00	1.42±0.03	1.45±0.00	1.47±0.03
Lactose (%)	1	4.16±0.04	4.59±0.96	3.57±0.07	2.97±0.26
	5	3.99±0.07	4.11±0.16	3.59±0.18	3.34±0.14
	10	4.03±0.22	4.10±0.12	3.63±0.15	3.60±0.05
Protein (%)	1	2.34±0.00	2.52±0.00	2.85±0.11	2.98±0.06
	5	2.38±0.59	2.43±0.01	2.94±0.00	2.99±0.01
	10	2.38±0.05	2.44±0.00	2.81±0.59	2.90±0.06
Titratable acidity (%)	1	0.82±0.01	0.75±0.01	0.77±0.02	0.74±0.00
	5	0.92±0.00	0.86±0.01	0.86±0.02	0.86±0.14
	10	0.91±0.02	0.87±0.01	0.92±0.00	0.86±0.18
pH	1	4.21±0.02	4.25±0.07	4.27±0.03	4.30±0.00
	5	4.12±0.02	4.22±0.02	4.23±0.04	4.30±0.00
	10	3.95±0.01	4.15±0.00	4.17±0.00	3.97±0.14
Acetaldehyde (ppm)	1	6.57±0.03	6.29±0.05	6.66±0.04	8.17±0.10
	5	8.81±0.11	9.11±0.04	9.64±0.08	8.92±0.10
	10	8.21±0.02	7.14±0.07	8.45±0.21	8.49±0.36
Tyrosine (mg/100mL)	1	0.12±0.00	0.12±0.00	0.11±0.00	0.12±0.00
	5	0.12±0.00	0.12±0.00	0.12±0.00	0.12±0.00
	10	0.13±0.00	0.12±0.00	0.12±0.00	0.13±0.00

FD1: Samples containing only yoghurt culture,
FD2: Samples containing *Str. thermophilus*, *Lb. bulgaricus*, *Lb. acidophilus* and *Bifidobacterium* spp.,
FD3: Samples containing *Str. thermophilus*, *Lb. lactis* and *Lb. acidophilus*,
FD4: Samples containing *Lb. casei* subsp. *rhamnosus* and yoghurt culture

growth and metabolism of selected strains of probiotic bacteria (*B. animalis* BB12, *Lb. rhamnosus GG*, *Lb. acidophilus* La5, *Lb. acidophilus* 1748 and *Lb. reuteri* SD 2112). The titratable acidity values were in an increasing attitude. In the same way, the pH values of the fermented milk samples were in a decreasing trend, as expected. There are various reports that describe the effect of pH on viability of probiotic bacteria in fermented milks. Special attention was attributed to post-acidification so that pH values do not fall below or probiotic viability could be affected (Shah *et al*., 1990; Rodas *et al*., 2002). Kailasapathy (2006) found 4.49 pH in the control group and 4.52 in the group containing probiotic bacteria, where he observed respectively 4.07 and 4.34 pH at the second week of the storage period.

The acetaldehyde values were found to have similarities with some other scientific studies. It was reported that 2.49-7.62 ppm of acetaldehyde contents in fermented milk samples after 60 days of storage (Atamer *et al*., 1999; Ozunlu, 2004). Moreover it was observed that 7.6-10.9 ppm of acetaldehyde after 15 days of storage by using different methods of production (Avsar *et al*., 2001; Ozunlu, 2004).

The tyrosine based spectrophotometric assay detects released free amino groups that result from the proteolysis of milk proteins, thus giving a direct measurement of proteolytic activity. Table 1 represents the proteolytic activities of lactic cultures. As seen that the tyrosine content of the samples was so close to each other and also it was found as 0.12 in almost all samples during the storage period. Being the proteolytic activity of probiotics low in normal and cold situations and low dry matter of milk are the reasons of low tyrosine levels. These phenomena could be explained as below.

Starter cultures containing *Lb. acidophilus*, *Bifidobacterium* spp. and *Lb. casei* subsp. *rhamnosus* have been becoming popular in the world. Dave and Shah (1997) observed a 3-4 log cycle drop in the counts of *Bifidobacterium spp* in ABT cultures. According to our results; rest of the strains used, did not release enough amount of free aminogroups in all samples ($p>0.05$), thus those strains could be classified as non proteolytic. This may explain why *Bifidobacteria* and *Lactobacillus* grow slowly in milk. It is assumed that free amino acids could be utilized during early stage of incubation and that peptides could become available during the prolonged incubation of *Bifidobacterium* and *Lactobacillus* cultures. This may also explain why the growth of probiotic bacteria requires supplementation of peptides and amino acids from external sources. Besides, it could be said that the differences of chemical characteristics of fermented milks are possibly due to difference of milk quality which depends on cow

Table 2: *Str. thermophilus* counts observed during the storage period in probiotic fermented milk samples (log cfu/mL, n = 2)

	Samples			
Days	FD1	FD2	FD3	FD4
1	8.70±0.01	7.35±0.07	8.55±0.07	8.47±0.10
5	8.80±0.09	7.38±0.04	8.66±0.07	8.60±0.00
10	8.69±0.06	7.30±0.00	8.60±0.01	8.47±0.03

FD1: Samples containing only yoghurt culture,
FD2: Samples containing *Str. thermophilus*, *Lb. bulgaricus*, *Lb. acidophilus* and *Bifidobacterium* spp.,
FD3: Samples containing *Str. thermophilus*, *Lb. lactis* and *Lb. acidophilus*,
FD4: Samples containing *Lb. casei* subsp. *rhamnosus* and yoghurt culture

Table 3: *Lb. bulgaricus* and *Lb. delbrueckii* spp. *lactis* counts observed during the storage period in probiotic fermented milk samples (log cfu/mL, n=2)

	Samples			
Days	FD1	FD2	FD3[1]	FD4
1	8.17±0.03	7.27±0.03	6.44±0.08	8.07±0.03
5	8.25±0.07	7.30±0.00	6.50±0.14	8.15±0.07
10	8.12±0.03	7.19±0.00	6.40±0.07	8.17±0.03

[1]Counts only for *Lactobacillus delbrueckii* spp. *lactis*,
FD1: Samples containing only yoghurt culture,
FD2: Samples containing *Str. thermophilus*, *Lb. bulgaricus*, *Lb. acidophilus* and *Bifidobacterium* spp.,
FD3: Samples containing *Str. thermophilus*, *Lb. lactis* and *Lb. acidophilus*,
FD4: Samples containing *Lb. casei* subsp. *rhamnosus* and yoghurt culture

Table 4: Probiotic bacteria counts of probiotic fermented milk samples (log cfu/mL, n = 2)

	Samples			
	FD2		FD3	FD4
Days	*Bifidobacterium* spp.	*Lb. acidophilus*	*Lb. acidophilus*	*Lb. rhamnosus*
1	5.09±0.12	4.00±0.14	8.18±0.21	6.42±0.35
5	5.06±0.19	4.15±0.07	8.22±0.35	6.45±0.07
10	4.98±0.23	4.10±0.21	8.20±0.14	6.37±0.10

FD2: Samples containing *Str. thermophilus*, *Lb. bulgaricus*, *Lb. acidophilus* and *Bifidobacterium* spp.,
FD3: Samples containing *Str. thermophilus*, *Lb. lactis* and *Lb. acidophilus*,
FD4: Samples containing *Lb. casei* subsp. *rhamnosus* and yoghurt culture

species, processing methods, starter culture types, incubation norms as well as probiotic microorganism species added in starter culture.

Microbiological properties

***Str. thermophilus* counts:** Table 2 shows the results of the microbiological counts for *Str. thermophilus* carried on the probiotic fermented milk samples. It was found that FD2 that contains *Bifidobacterium spp.* and *Lb. acidophilus* had lower viable cells of *Str. thermophilus* than the other three products. The highest *Str. thermophilus* count during the storage period was found to be the control group.

***Lb. bulgaricus* and *Lb. lactis* counts:** Table 3 shows the results of the microbiological counts for *Lb. bulgaricus* carried on the probiotic fermented milk samples. The viable cell counts of *Lb. bulgaricus* and *Lb. lactis* in FD2 and FD3 samples were observed to have a lower population than the other samples. Also, in FD3 sample the viable counts of *Lb. lactis* were lower than the other samples that contain *Lb. bulgaricus*.

Probiotic bacteria counts: Viability of probiotic bacteria of fermented milk samples are presented in Table 4. The viable cell counts of *Bifidobacterium* spp. were 4.98-5.09 log cfu/mL whereas the *Lb. acidophilus* counts were between 4.00-4.15 log cfu/mL in sample FD2. *Lb. acidophilus* population at the end of the storage period was found to be higher than the initial population. *Lb. acidophilus* counts were found between 8.18-8.22 log cfu/mL in sample FD3. Compared to FD2 sample, it was found that *Lb. acidophilus* population was found higher in FD3 sample. The viable cell counts of *Lb. casei* subsp. *rhamnosus* were between 6.37 and 6.42 log cfu/mL in sample FD4.

Sensory analysis: The sensory qualities of the experimental fermented milks were assessed by the scaling method. Appropiately trained and prepared panel of 6 judges analyzed the sensory profile of the fermented milks. The assessment was carried out at the Dairy Technology Department of the Agriculture Faculty of the Ege University in izmir which fulfills appropriate requirements of the International Standard Organization. In the initial evaluation the panel selected its own set of describing properties of the assessed product. The intensity of each of characteristics was evaluated in a ten point score in accordance with the descriptive values 1-absence 10-very clear. The following characteristics were assessed bitten taste, yeasty taste, fermented taste, sour taste, fermented odor, sour odor, viscosity, astringency, appearance, serum separation and overall acceptability (Cais-Sokolinska *et al*., 2008).

Changes in the acidity and the content of taste and odor compounds in the examined fermented milks were accompanied by changes in the quality characteristics assessed organoleptically. In the study; the type of applied probiotic culture and storage time exerted a significant effect on the results of the sensory evaluation of its overall acceptability. The type of the applied starter culture on the fermented milks quality parameters depended on the time of storage generally. The analyzed fermented milks were found more desirable by the panelists after 5 days of storage and producing. The lowest scores for the overall acceptability were given to the fermented milk samples evaluated after 10 days of storage.

It was indicated that all three samples containing probiotic bacteria had higher points than the control sample. The probiotic fermented milk product that contains *Lb. acidophilus* and *Bifidobacterium* spp. (FD2)

Table 5: Sensory analysis results of probiotic fermented milk samples (n = 2)

		Samples			
	Days	FD1	FD2	FD3	FD4
Odor and Flavor	1	6.80±1.13	7.15±1.20	7.45±0.21	7.30±0.42
	5	6.08±0.35	7.60±0.00	7.33±0.46	7.23±0.09
	10	5.87±0.17	7.00±0.00	6.25±0.35	6.50±0.35
Consistency	1	7.80±0.28	6.60±0.00	7.45±0.21	6.80±0.70
	5	6.99±0.23	7.56±0.37	7.33±0.00	7.25±0.35
	10	6.50±0.00	7.37±0.17	7.00±0.70	6.87±0.53
General	1	7.30±0.00	7.30±0.42	7.65±0.49	7.45±0.21
	5	5.83±0.00	7.66±0.23	7.49±0.23	7.41±0.12
	10	6.00±0.00	7.00±0.00	6.25±0.35	6.87±0.17

FD1: Samples containing only yoghurt culture,
FD2: Samples containing *Str. thermophilus*, *Lb. bulgaricus*, *Lb. acidophilus* and *Bifidobacterium* spp.,
FD3: Samples containing *Str. thermophilus*, *Lb. lactis* and *Lb. acidophilus*,
FD4: Samples containing *Lb. casei* subsp. *rhamnosus* and yoghurt culture

was the most liked for odor and flavor evaluation. Furthermore, the panelists evaluated the products for their aroma/flavor, consistency and overall acceptability. Thus, sample FD2 was again the most liked product among the products. To make a general statement, the majority of the panelists in the sensory evaluation panels described the probiotic fermented milk samples as having better taste and flavour and spoke of a better durability of taste, flavour and acidity until the end of the storage period when compared to the control product.

Conclusions: As a result it could be said that acceptable fermented milk, when compared with a traditional yoghurt drink (ayran), had been obtained by supplementation of yoghurt culture with probiotic bacteria. It was shown that main advantages of probiotic addition to traditional yoghurt culture were a reduction in fermentation time, nutritional bioavailability of the product, an increase in probiotic bacteria growth and viability and the development of suitable chemical, microbiological and sensory properties for a probiotic milk drink. It could be said that addition of probiotic bacteria neither did adversely affect the acceptability of the products that had a high score for odor, flavor, consistency and general acceptance, nor did it cause acidification during storage.

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