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Evaluation of Some Probiotic Fermented Milk Products From Al-Ahsa Markets, Saudi Arabia

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Abstract: Eight commercial probiotic fermented milk products (six full fat and two low fat) from Al-Ahsa markets were evaluated for chemical, microbiological and sensory properties. The chemical composition parameters ranged from 0.9-1.2% fat (low fat products), 3.0-3.9% fat (full fat products), 3.1-4.7% protein, 0.7-1.2% ash and 7.5-3.7% carbohydrate in all the milk products. The pH values of all the products decreased significantly from the production day to the end of storage period. With respect to the microbiological side, the coliform bacteria, moulds and yeasts counts were not detected in all the products during the refrigerated storage at 5±1°C. However, seven out of eight products contained over 10^6 cfu mL^{-1} of bifidobacteria on the production day. Only two of these products maintained 10^6 cfu mL^{-1} viable count of bifidobacteria till the end of cold storage period. On the other hand, three out of eight products showed the highest number of *L. acidophilus* viable count (above 10^8 cfu mL^{-1}) on production day. The results of sensory evaluation showed that all the tested products obtained high scores for flavor, appearance, texture or consistency and smell (odor) properties during the storage period. These results suggest that for optimum benefits, the probiotic fermented milk products with live probiotic bacteria should be consumed within one week of their production date. The research provided useful information to the dairy industries to develop new technology to ensure the supply of high quality milk products to the consumers.

Key words: Probiotic milk products, fat, protein, ash, carbohydrates, high quality products, bifidobacteria

INTRODUCTION

Generally, the fermented dairy products are considered safe and nutritious. The beneficial effects of fermented milk products may be further enhanced by supplementation of probiotic bacteria such as *Lactobacillus* and *Bifidobacterium* species. A probiotic is generally defined as a live microbial supplement which beneficially affects the host animal by improving its intestinal microbial balance (Fuller, 1989). Several health benefits are related to the regular consumption of viable probiotic bacteria including improvement of lactose tolerance (Kim and Gilliland, 1983) antimicrobial (Yildirim and Johnson, 1998), anti-carcinogenic (Abd El-Gawada *et al*., 2004), hypocholesterolemic (Kikuchi-Hayakawa *et al*., 2000; Abd El-Gawada *et al*., 2005) and anti-mutagenic (Hsieh and Chou, 2006). Fermented dairy products are considered as vehicles by which the consumers might receive adequate numbers of probiotic bacteria (Samona and Robinson, 1994; Stanton *et al*., 1998). In order to produce therapeutic benefits, sufficient number of viable microorganisms must be present throughout the entire shelf life of the product. In this regard, the ranges of minimum levels for probiotic bacteria in fermented milks were suggested from 10^5-10^6 cfu mL^{-1} (Samona and Robinson, 1994). Schuller-Malyoth *et al*.

(1968) reported that a good probiotic culture should contain between 10^6 and 10^8 viable cells per milliliter. For bifidobacteria to provide therapeutic benefits, it was recommended that they must be viable and ingested in numbers $\geq 10^6$ cells per gram of yoghurt (Kurman and Rasic, 1991). Therefore, maintaining the viability of probiotic bacteria until the products are consumed is of great interest for the dairy industries. Several factors such as acidity, pH, hydrogen peroxide, oxygen content, temperature of storage at the time of production and the storage facilities of fermented milk affect the viability of probiotic bacteria in yoghurt (Samona and Robinson, 1994; Lankaputhra and Shah, 1995; Lankaputhra *et al*., 1996). Recently, a wide variety of probiotic fermented milk products are commercially available in the Saudi Arabia. These products are exposed to high temperature during handling and transportation and to cold storage in the markets for minimum one week until consumption in Saudi Arabia. The aim of this study was to determine the viable count of probiotic bacteria (*Lactobacillus acidophilus* and bifidobacteria) in some probiotic fermented milk products from Al-Ahsa markets during refrigerated storage and also to assess the effect of pH and cold storage period at 5±1°C on the viability of probiotic bacteria in these milk products.

MATERIALS AND METHODS

Collection of Fermented Milk Products

Eight commercial probiotic fermented milk products (six full fat and two low fat) were collected on production day from Al-Ahsa markets, Saudi Arabia during 2007. These samples were analyzed microbiologically on the production day and then every week for three weeks during refrigerated storage at 5±1°C. The same samples were stored in the refrigerator for chemical analysis. A brief description of these products and their labeled ingredients are shown in Table 1.

Analytical Methods

Chemical Composition

Moisture contents of probiotic fermented milk products was determined according to the Association of Official Analytical Chemists method (AOAC, 1995a). Total Nitrogen (TN) was determined by Kjeldahl Method (AOAC, 1995b) and fat content by the Gerber method as described in the British Standard method (1989). The ash content was determined according to the AOAC method (AOAC, 1995c).

Total carbohydrate was calculated by difference as follows (Manzi *et al*., 2007):

$$\text{Carbohydrate (g/100 g)} = 100-(\text{Water}+\text{Protein}+\text{Fat}+\text{Ash})$$

Total energy was calculated according to the following equation (CEE Directive, 1990):

$$\text{Energy (kcal/100 g)} = 4\times(\text{g protein}+\text{g carbohydrate})+9\times(\text{g lipid})$$

Table 1: Description of the commercial probiotic fermented milk products

Product	Description
A	Stirred yoghurt (fresh milk 50%, recombined milk 50%, fresh cream, contains Bifidobacteria)
B	Laban (fresh milk 50%, recombined milk 50%, contains Bifidobacteria)
C	Laban (100% fresh cow milk, contains Bifidobacteria and *Lactobacillus acidophilus*)
D	Laban low fat (100% cow milk, contains Bifidobacteria and *Lactobacillus acidophilus*)
E	Laban (cow milk, contains probiotic culture)
F	Laban low fat (cow milk, contains probiotic culture)
G	Set yoghurt (fresh cow milk 50%, recombined milk 50% fresh cream, contains Bifidobacteria)
H	Laban (fresh milk 100%, contains *Lactobacillus helveticus*, Bifidobacteria, *Lactobacillus acidophilus*)

pH Determination

The pH of probiotic fermented milk products was measured with a digital pH meter by using a TPS digital pH meter (Denver Instruments, TX, USA). The pH of yoghurt samples was determined by direct immersion of the electrode into the sample (20-25 mL) at room temperature.

Coliform Bacteria

The count of coliform group was estimated by plating on McConkey agar medium (Oxid), as recommended by the APHA (1992). The plats were incubated at 37°C for 2 days.

Moulds and Yeasts

Potato dextrose agar medium (Oxid) was used for enumerating yeasts and moulds count according to APHA (1992). The plats were incubated at 20-25°C for 2-3 days.

Bifidobacteria Estimation

The count of bifidobacteria was enumerated according to the method of Dinakar and Mistry (1994), in which a mixture of antibiotics, including 2 g paromomycin sulphate, 0.3 g nalidixic acid and 60 g lithium chloride, was dissolved in 1 L distilled water, filter-sterilized (0.2 μm) and stored at 4°C until use. The antibiotic mixture (5 mL) was added to 100 mL MRS-agar medium. L-Cysteine-HCl 0.5% (w/v) (Sigma Chemical Co., St. Louis, MO, USA) was also added to decrease the redox potential of the medium. Plates were incubated at 37°C for 48 h an-aerobically.

Lactobacillus acidophilus

The count of *Lactobacillus acidophilus* was determined according to Van de Casteele *et al*. (2006) by using MRS medium + 0.5 ppm filter sterilized clindamycin. Plates were incubated at 37°C for 48 h an-aerobically.

Sensory Evaluation

Sensory evaluation of commercial probiotic fermented milk products was carried out on the production day and for 3 weeks of cold storage using a regular score panel according to Tamime and Robinson (1985).

RESULTS AND DISCUSSION

Chemical Composition

The results showed great variability in the chemical composition of different fermented milk products (Table 2). The ranges of various chemical components were 0.9-3.9% fat, 3.1-4.7% protein, 0.7-1.2% ash and 7.5-3.7% carbohydrate. Low fat products such as D and F were high in water content and less total energy than other products. However, product A showed high protein and carbohydrate contents and less moisture content than other tested products thus showing more energy. Whereas,

Table 2: Chemical composition (g/100 g) of commercial probiotic fermented milk products[a]

Products[b]	Moisture	Protein	Fat	Ash	Carbohydrate	Energy kcal/100 g
A	82.9±0.2	4.7±0.2	3.8±0.1	1.1±0.0	7.5	83.0
B	88.4±0.0	3.1±0.1	3.9±0.1	0.7±0.0	3.9	63.1
C	88.6±0.0	3.3±0.1	3.5±0.1	0.7±0.0	3.9	42.8
D	90.7±0.0	3.4±0.0	1.2±0.1	1.0±0.0	3.7	39.2
E	88.1±0.4	3.1±0.1	3.9±0.1	0.7±0.0	4.2	64.3
F	90.6±0.0	3.8±0.6	0.9±0.1	0.7±0.1	4.0	39.3
G	85.6±0.2	4.0±0.4	3.0±0.1	1.2±0.1	6.2	67.8
H	88.5±0.1	3.3±0.1	3.1±0.1	0.7±0.0	4.4	58.7

[a]Analytical data are means of triplicate analysis standard deviation, [b]Product samples as in Table 1

Table 3: Changes in pH values of probiotic fermented milk products during refrigerated storage period 5±1°C

	pH values during storage period (weeks)			
Products*	0#	1	2	3
A	4.64±0.01[a]	4.63±0.00[a]	4.60±0.01[b]	4.46±0.01[c]
B	4.56±0.01[a]	4.59±0.01[a]	4.56±0.02[a]	4.34±0.01[b]
C	4.43±0.01[a]	4.39±0.01[a]	4.28±0.01[b]	4.27±0.03[b]
D	4.46±0.01[a]	4.42±0.01[b]	4.41±0.01[b]	4.32±0.02[c]
E	4.44±0.01[a]	4.40±0.01[b]	4.36±0.01[c]	4.35±0.01[c]
F	4.49±0.02[a]	4.41±0.01[b]	4.40±0.00[b]	4.35±0.03[c]
G	4.76±0.01[a]	4.70±0.01[b]	4.68±0.01[b]	4.58±0.01[c]
H	4.45±0.01[a]	4.39±0.01[b]	4.35±0.01[c]	3.30±0.01[d]

[a-d]Mean values (±SD; n = 3) in row with the same letter(s) are not significantly different from each other at $p>0.05$, *Product samples as in Table 1, #Product in one-day old

the fat contents of B and E products were higher than other products (3.9%). Whereas, G, A and D products were higher in ash contents when compared with the other products. In Italian market, Manzi *et al*. (2007) reported that the fat, protein and ash content of probiotic fermented milk ranged from 0.2-3.6, 2.7-5.8 and 0.4-0.8%, respectively.

Changes in pH Values

The initial pH values of fermented products ranged from 4.43-4.76. In general, the pH of all the tested products decreased gradually from the production day to the end of storage period (Table 3). The difference in pH was significant between the production day and the end of storage period. There were no significant differences in pH values among A, B and C products between the production day and the first week of storage. However, H product showed significant decrease in pH (3.30) which could be due to the presence of three strains of starter *Lactobacillus helveticus*, Bifidobacteria and *Lactobacillus acidophilus* (Table 1) compared with other products. Shah *et al*. (1995) also found similar decreases in pH values during storage of commercial yoghurts containing *L. acidophilus* and *B. bifidum*. Similarly, the initial pH values in yoghurts containing *L. acidophilus* and bifidobacteria decreased from 4.33-4.41 at day 0 to 4.16-4.22 at the end of 35 days of storage (Dave and Shah, 1997). In parallel, Akalin *et al*. (2004) found the initial pH values of different types of yoghurt in the range of 4.51-4.48 which slightly decreased during storage.

Coliform Bacteria, Moulds and Yeasts

The coliform bacteria and moulds and yeasts counts were not detected in all tested products on the production day and during the refrigerated storage at 5±1°C for 3 weeks. This could be attributed to the high hygienic systems implemented in these factories.

Survival of Bifidobacteria

The populations of bifidobacteria decreased significantly from the production day to the end of refrigerated storage period at 5±1°C in all the commercial probiotic fermented milk products (Table 4). However, during the two weeks of cold storage, the population of bifidobacteria remained above 10^5 cfu mL^{-1}. The viability losses of bifidobacteria showed between 0.5 and 3.74 log cycles in F and D products, respectively. Interestingly, the G product recorded maximum viable count of bifidobacteria on the production day as well as at the end of storage (9.88 and 6.71 log cfu mL^{-1}, respectively) than all the other products. This could be due to the highest pH value of G product than other products (Table 3). Whereas, F product showed minimum count of this organism. According to Kurman and Rasic (1991), the viable level of bifidobacteria must be above 10^6 cfu mL^{-1} to provide therapeutic benefits. Among the various products, B and G fulfilled this requirement for viability of bifidobacteria till the end of cold storage period. The reduction of bifidobacterial count may be due to

Table 4: Survival (log cfu mL^{-1}) of bifidobacteria in commercial probiotic fermented milk products during refrigerated storage

Products*	Count of bifidobacteria during storage period (weeks) 0#	1	2	3	Log reduction
A	8.36±0.13^{a}	7.18±0.01^{b}	6.41±0.30^{c}	5.42±0.33^{d}	2.94
B	7.57±0.10^{a}	7.50±0.14^{a}	6.44±0.11^{b}	6.38±0.11^{b}	1.19
C	7.49±0.01^{a}	6.78±0.08^{b}	5.65±0.35^{c}	4.28±0.04^{d}	3.21
D	7.88±0.01^{a}	6.14±0.01^{b}	5.68±0.39^{b}	4.14±0.02^{c}	3.74
E	7.21±0.15^{a}	6.18±0.40^{b}	5.65±0.13^{bc}	5.41±0.13^{c}	1.80
F	5.34±0.03^{a}	5.17±0.06^{a}	5.35±0.35^{a}	4.84±0.12^{a}	0.50
G	9.88±0.04^{a}	9.35±0.30^{a}	6.93±0.59^{b}	6.71±0.56^{b}	3.17
H	7.43±0.22^{a}	7.26±0.07^{a}	6.04±0.25^{b}	4.83±0.06^{c}	2.60

[a-d]Mean values (±SD; n = 3) in row with the same letter(s) are not significantly different from each other at p>0.05, *Product samples as in Table 1, #Product in one-day old

Table 5: Survival (log cfu mL^{-1}) of *Lactobacillus acidophilus* in commercial probiotic fermented milk products during refrigerated storage

Products*	Count of *Lactobacillus acidophilus* during storage period (weeks) 0#	1	2	3
A	8.66±0.01^{a}	7.40±0.42^{b}	6.86±0.36^{bc}	6.26±0.21^{c}
B	8.66±0.01^{a}	7.52±0.54^{ab}	6.44±0.37^{bc}	6.10±0.71^{c}
C	5.20±0.14^{a}	4.45±0.21^{b}	4.10±0.28^{bc}	3.75±0.21^{c}
D	5.72±0.68^{a}	4.67±0.16^{b}	4.55±0.07^{b}	3.74±0.23^{b}
E	6.54±0.06^{a}	5.77±0.23^{b}	5.35±0.22^{bc}	5.15±0.06^{c}
F	5.76±1.17^{a}	4.97±0.19^{a}	4.76±0.06^{a}	4.55±0.03^{a}
G	9.53±0.04^{a}	7.34±0.32^{b}	6.62±0.69^{b}	6.40±0.14^{b}
H	6.42±0.07^{a}	6.21±0.04^{a}	5.17±0.01^{b}	4.08±0.18^{c}

[a-d]Mean values (±SD; n = 3) in row with the same letter(s) are not significantly different from each other at p>0.05, *Product samples as in Table 1, #Product in one-day old

the decrease of pH values, post process acid production (Wang *et al*., 2002), sensitivity to oxygen (Shimamura *et al*., 1992), metabolites such as hydrogen peroxide and ethanol and to bacteriocins produced by lactic acid bacteria (Frank and Marth, 1988). These results were in agreement with those of (Samona and Robinson, 1994; Medina and Jordano, 1994; Lankaputhra and Shah, 1995; Lankaputhra *et al*., 1996), who found poor viability of bifidobacteria in yoghurt during storage.

Survival of *Lactobacillus acidophilus*

Generally, the viability of *L. acidophilus* decreased gradually and significantly in commercial probiotic fermented milk products during cold storage (Table 5). Among all the products, A, B and G recorded the highest number of *L. acidophilus* viable count (8.66, 8.66 and 9.53 log cfu mL^{-1}, respectively) on the production day. On the other hand, the C, D and F products showed minimum count of this organism. During storage period, the viable count of the bacteria ranged between 3.7 and 7.5 log cfu mL^{-1} in all the products. Speck (1976) reported that 10^8 to 10^9 viable cells of *L. acidophilus* should be ingested daily to ensure that consumers receive health benefits. Among all the products A, B and G fulfilled this requirement for viability of *L. acidophilus* on the production day. The reduction in the count of *L. acidophilus* during the cold storage may be due to the production of antimicrobials such as bacteriocins, H_2O_2, or organic acids. Characterizing bacteriocins and bacteriocin-like inhibitory substances produced by *L. acidophilus* and other lactic-acid bacteria were reported by Shah and Dave (2002). These results agree with those of Shah *et al*. (1995), who reported that three out of five brands of fresh yogurt contained 10^7 to 10^8 viable cells of *L. acidophilus* per gram while the remaining two brands contained less than 10^5 *L. acidophilus* cells per gram. Nighswonger *et al*. (1996) found that some strains of *L. acidophilus* lost viability during storage at 7°C after 28 days. Dave and Shah (1997) found that the survival of *L. acidophilus* in yogurts after 35 days of storage was

Table 6: Sensory evaluation of commercial probiotic fermented milk products during storage period

	Flavor point (50)				Appearance point (20)			
	Storage period (week)				Storage period (week)			
Products*	0¥	1	2	3	0	1	2	3
A	44.0±4.0a	43.4±3.0a	43.4±6.4a	36.4±3.0b	17.4±1.2a	16.0±3.4a	17.4±3.0a	16.0±1.0a
B	40.6±3.2a	44.0±2.0a	41.4±6.4a	35.6±4.2b	16.6±1.2a	17.4±1.2a	14.0±5.2a	15.4±2.4a
C	42.0±5.4a	42.6±4.2a	40.6±5.0a	39.4±4.2a	14.6±1.2a	13.4±2.4a	14.6±3.0a	13.4±3.0a
D	42.0±5.2a	44.0±2.0a	42.6±3.0a	40.0±2.0a	16.0±0.0a	16.0±0.0a	15.4±3.0a	13.4±1.2a
E	46.0±2.0a	44.6±4.2a	44.0±5.2a	35.6±4.2b	17.4±1.2a	17.4±1.2a	16.0±3.4a	14.6±2.4a
F	45.4±2.6a	42.6±6.4a	43.4±5.0a	42.0±4.0a	18.0±0.0a	12.6±6.4a	15.4±4.6a	14.0±3.4a
G	45.4±1.2a	44.0±4.0a	43.4±6.4a	33.0±5.2b	17.4±3.0a	16.6±1.2a	16.0±2.4a	16.0±2.0a
H	42.0±5.2a	44.0±4.0a	42.6±4.2a	39.4±4.2a	16.0±0.0a	16.0±3.4a	15.4±4.6a	14.0±2.0a

	Texture or consistency point (20)				Smell or odor point (10)				Total point (100)			
	Storage period (week)				Storage period (week)				Storage period (week)			
Products*	0	1	2	3	0	1	2	3	0	1	2	3
A	17.4±1.2a	16.6±1.2a	17.4±3.0a	16.0±2.0a	8.6±1.2a	8.0±0.0a	7.4±1.2a	7.4±1.2a	87.4	84.0	85.6	75.8
B	16.0±2.0a	16.0±2.0a	15.2±2.4a	14.0±2.0a	9.4±1.2a	8.6±1.2a	8.0±0.0a	8.0±0.0a	80.6	86.0	78.6	73.0
C	16.0±2.4a	14.0±2.0a	15.2±1.2a	14.0±2.0a	7.4±1.4a	7.4±1.2a	6.4±1.2a	6.0±2.0a	80.0	77.4	75.0	72.8
D	16.0±2.4a	16.0±2.0a	15.4±2.4a	14.6±1.2a	90±1.0a	8.0±0.0a	8.0±0.0a	6.6±2.4a	83.0	84.0	81.4	74.6
E	16.6±3.2a	17.4±1.2a	16.6±2.4a	15.4±1.2a	8.6±1.2a	8.0±0.0a	8.0±2.0a	7.4±1.2a	88.6	87.4	84.6	73.0
F	18.0±0.0a	18.0±2.0a	16.0±3.4a	14.6±2.4a	8.0±2.0a	7.4±1.2a	7.4±1.2a	7.4±1.2a	89.4	80.6	82.2	78.0
G	16.6±2.4a	16.6±1.2a	17.4±3.0a	16.6±2.4a	8.0±0.0a	8.0±0.0a	8.0±2.0a	7.4±1.2a	87.4	85.2	84.8	73.0
H	17.4±3.0a	16.0±2.4a	15.4±2.4a	14.6±1.2a	9.0±1.0a	8.6±1.2a	8.0±0.0a	8.0±0.0a	84.4	84.0	81.4	68.1

$^{a-d}$Mean values (±SD; n = 3) in row with the same letter(s) are not significantly different from each other at p>0.05, *Product samples as in Table 1, ¥Product in one-day old

approximately 0.1 to 5% as compared to after 5 days of storage. Olson and Aryana (2008) found that the *L. acidophilus* counts in yogurt tended to decrease from 6.84 to 4.43 log cycles during 8 week of storage time.

Sensory Evaluation

All the tested products scored high points in flavor, appearance, texture or consistency and smell (odor) during the refrigerated storage period (Table 6). However, there was no significant difference (p>0.05) in the appearance, body and texture and smell (odor) of all tested products during storage period. With respect to the flavor score, the A, B, E and G products showed significant differences in the 3rd week of storage period.

Formation of exopolysaccharide by the starter and probiotic cultures may contribute to prevention of synersis and an increase in viscosity, combined with a better mouth feel (Hussein *et al*., 1996). Griffin *et al*. (1996) reported that polysaccharide producing yoghurt bacteria were important determinants of yoghurt viscosity and texture. These starter cultures improve the viscosity of yoghurt leading to resistance to mechanical damage (Tamime and Deeth, 1980).

CONCLUSIONS

Seven out of eight products contained over 10^6 cfu mL^{-1} of bifidobacteria on the production day. Only two of these products maintained 10^6 cfu mL^{-1} viable count of bifidobacteria till the end of cold storage period. Three out of eight products showed the highest number of *L. acidophilus* viable count (above 10^8 cfu mL^{-1}) on production day. The sensory evaluation showed that all the tested products obtained high scores for flavor, appearance, texture or consistency and smell (odor) properties during the storage period. These results suggest that to obtain optimum health benefits, the probiotic fermented milk products with live probiotic bacteria should be consumed within one week of their production date. The research provided useful information to the dairy industries to develop new technology to ensure the supply of high quality milk products to the consumers.

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