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Viability of Probiotic Bacteria during Refrigerated Storage of Commercial Probiotic Fermented Dairy Products Marketed In Jordan

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Abstract

Objectives: The study aimed to measure the viability of probiotic bacteria in different probiotic fermented dairy products marketed in Jordan during their shelf lives.

Methods: Ten products which were all known commercial probiotic fermented dairy drinks were purchased from main market stores at 0 day of processing, and kept under 4^{o}C for the assigned time intervals (1, 7, 14 day). These products included 7 stirred yogurt Activia, Activia low-fat, Actimel, Baladna, Acti-Yogho, Moffedo, and Vital, and 2 set yogurt (Activia - apricot and peach, Activia light – strawberry) and one stirred yogurt (Activia-stirred). Samples were tested for probiotic count at those intervals in an unopened refrigerated bottles. Sensory evaluation using hedonic scale was carried out on the above products in an unopened package at the same indicated intervals. Morphology of probiotic bacteria in commercial products was also confirmed microscopically.

Results: The results of the viability of probiotic counts in $\log_{10}$ remaining above 7 log until the end of shelf life (14 d) except for four products including Moffedo, vital, Activia set yogurt light – strawberry, and Activia (stirred yogurt) which decreased to 3.4, 4.9, 5.0, and 5.0 respectively at the end storage period. The pH for all products until the end of the study were between 4.1- 4.5. The best average of all sensory characteristics using hedonic scale (8.3) was for Actimel, whereas the lowest (7.1) was for both Moffedo and Activia set yogurt-Apricot and peach.

Conclusion: The counts of probiotic bacteria in fermented dairy products is not always above the therapeutic dose of 6.0 log cfu/g, which urge governmental authorities to establish a standard related to these products.

Keywords: probiotic bacteria, fermented dairy products, viability, bifidobacteria, lactic acid bacteria, Jordan

1. Introduction

Functional foods are ones that contain chemical and/or microbial components that may affect beneficially one or more target functions in the body, beyond adequate nutritional effects, in a way that is relevant to either the state of well-being and health or the reduction of the risk of a disease (Diplock et al., 1999). The term probiotic is a relatively new word meaning “for life” and it is currently used to name bacteria associated with beneficial effects for humans and animals (FAO/WHO, 2002). Probiotic food products are classified as a functional food and represent a significant part of the market that probiotic foods comprise between 60 and 70% of the total functional food market (Holzapfel, 2006). Today, a total of 78% of current probiotic sales in the world are delivered through yogurt fruit juices desserts and cereal-based products featuring probiotics may be other suitable media for delivering probiotics (Granato *et al*., 2010)

Many microorganisms have been used or considered for use as probiotics. A probiotic preparation may contain one or several different strains of microorganisms. Because viable and biologically active microorganisms are usually required at the target site in the host, it is essential that the probiotic be able to withstand the host’s natural barriers against ingested bacteria (Lankaputhra & Shah, 1995). The most commonly used probiotics are strains of lactic acid bacteria (e.g., *Lactobacillus*, *Bifidobacterium* and *Streptococcus*). The beneficial effects of *Lactobacillus* and *Bifidobacterium* have been discussed for decades. Bacteria in these two genera resist gastric acid, bile salts and pancreatic enzymes, adhere to intestinal mucosa and readily colonize the intestinal tract

(Kailasapathy & Rybka, 1997).

These species are known for their additional health benefits. These additional health benefits are as the companies using probiotic acclaim to decrease the rate of occurrence of diarrhea, infections, inflammations, colitis and irritable bowel syndrome, reduce blood serum cholesterol, help to reduce lactose intolerance and increase production of vitamins (Kneifel &Pacher, 1993). Lactic acid bacteria have been demonstrated to inhibit the in vitro growth of many enteric pathogens including *Salmonella typhimurium*, *Staphylococcus aureus*, *Escherichia coli*, *Clostridium perfringens* and *Clostridium difficile* and have been used in both humans and animals to treat a broad range of gastrointestinal disorders (Meurman *et al*. 1995).

A number of factors have been claimed to affect the viability of probiotic bacteria in yogurt, including acid and hydrogen peroxide produced by yogurt bacteria, oxygen content in the product, and oxygen permeation through the package (Lankaputhra and Shah, 1994). Although *L. acidophilus* and bifidobacteria tolerate acid, a rapid decline in their numbers in yogurt has been observed (Shah and Jelen, 1990).

Bifidobacteria are not as acid tolerant as *L. acidophilus*; the growth of the latter organisms ceases below pH 4.0, while the growth of the *Bifidobacterium* spp. is retarded below pH 5.0 (Shah, 1997).

These benefits are caused by the ability of probiotic bacteria to support the growth of intestinal micro flora to reach higher population than already existing, and so may inhibit pathogens. In order for the consumer of probiotic products to gain any health benefits, the product must have a minimum number of viable microorganisms throughout the shelf life of the product, and this minimum count is 10^6 colony forming unit (cfu)/ml (Samona & Robinson 1994). Another feature of interest is the identity of bacteria found in the products, so to make sure that the starter culture stated on bottles is used by phenotypic identification of probiotic bacteria is done (Temmerman, *et al*, 2003). The study aims to measure the viability of probiotic bacteria in different probiotic fermented dairy products marketed in Jordan during their shelf lives compared to the international products. It also helpful in evaluating the organoleptic acceptance of fermented probiotic dairy products marketed in Jordan.

2. Materials and Methods

2.1 Samples Collection

Ten products of probiotic dairy (all known commercial probiotic fermented dairy products marketed in Jordan) were purchased from main market stores at 0 day of processing (in the same production date printed on the label). The products were kept under refrigerated conditions during transportation of two hours (using ice box of about 5-8^o C) and directly stored in the laboratory at 4^{o}C at assigned time intervals (1, 7, 14 day). The names of tested products, companies and countries of manufacture are listed in Table 1. The products were included drinking yogurt (7trade names), set yogurt (2 trade names) and stirred yogurt (1 trade name). Samples were tested for probiotic count at the previous intervals in unopened refrigerated bottles.

Table 1. Commercial Probiotic fermented dairy products marketed in Jordan

No	Name Of Company	Name Of Product	Origin
1	Al-Safi Danone	Activia (yogurt drink)	Saudi Arabia
2	Al-Safi Danone	Activia low-fat (yogurt drink)	Saudi Arabia
3	Al-Safi Danone	Actimel (yogurt drink)	Saudi Arabia
4	Baladna Danish Jordanian Dairy Inc.	Yogurt Strawberry with pieces (yogurt drink)	Jordan
5	Hammodeh	Acti-Yogho (yogurt drink)	Jordan
6	Teeba-Almarai Group	Vital (yogurt drink)	Jordan
7	Haritna Dairy Company	Moffedo (yogurt drink)	Jordan
8	Al-Safi Danone	Activia set yogurt- Apricot and peach	Saudi Arabia
9	Al-Safi Danone	Activia set yogurt light – strawberry	Saudi Arabia
10	Al-Safi Danone	Activia (stirred yogurt)	Saudi Arabia

2.2 Probiotic Count

In order for the consumer of probiotic products to gain any health benefits, the product must have a minimum number of viable microorganisms throughout the shelf life of the product, and this minimum count is 10^6 colony forming unit (cfu)/ml (Samona & Robinson 1994). De-Man Rogosa Sharpe (MRS) media used in our work was prepared according to manufacturer directions (Oxoid,UK). The media was enriched with 0.05% L-Cysteine. Each product was homogenized properly before opening and one ml was taken by micropipette. Appropriate

serial dilutions were made up to 10^{-7} aseptically and each dilution was homogenized using the vortex. Pour plate method was used for probiotic count in duplicate. Plates were incubated anaerobically at $37 \pm 1^{o}C$ for 48 ± 2 hours, and numbers were expressed in CFU/ml. (Tharmaraj and Shah, 2003)

2.2.1 Morphology of Probiotic Bacteria in Commercial Products

All probiotic products were confirmed and tested for purity before counting by examining films of its cultures after staining by methylene blue (Loffler's methylene blue 0.002 %, as performed by Cowan and Steel, 1993).

2.3 Organoleptic Evaluation of Commercial Probiotic Fermented Dairy Products

Sensory evaluation was carried out on the previous samples of probiotic fermented dairy products collected from the market in an unopened package at assigned time intervals (1, 7, 14 day). Hedonic scale (a term used in tasting panels where the judges indicate the extent of their like or dislike for the food (Bender, 2014)) of 9 points of five sensory attributes (appearance, smell, taste, consistency, and overall acceptability) was used for the evaluation of the products. Twelve trained panelists distributed by 6 males and 6 females (include University students already trained in nutrition and food processing department through graduation project course) were participated in the evaluation (Clark *et al*., 2009). The dairy samples were served at room temperature, and they asked to put a mark from 9 which represent 'like extremely' to 1 which represent 'dislike extremely'

2.4 Statistical Analysis

SAS package system version 9.2 was used to analyze our results. t-test (LSD) was used to compare the probiotic count between the probiotic dairy products marketed in Jordan. t-test (LSD) was also used to compare the sensory tests of all evaluated products.

3. Results

Probiotic fermented dairy products in Jordan markets were evaluated considering the viability of probiotic counts and the organoleptic properties. The microscopic examination of the probiotic products indicates that the morphology is similar to the bifidobacteria (branched rod-shaped bacterium with a club shaped) and the morphology of *lactobacillus lacis.*

3.1 Probiotic Bacteria Counts

The viability of probiotic bacteria of seven drinking yogurts, two set yogurt samples and one of stirred yogurt were evaluated at 3 time intervals (1day, 7days, and 14 days) of shelf life. The results of the viability of probiotic previous products shown in Table 2.

Table 2. Counts of probiotic bacteria in the probiotic-fermented dairy products

Name of products	Days in shelf life	Log of average count	± Std Error	pH
	1	8.5*** ±	0.088	4.5
Activia	7	7.5*** ±	0.088	4.4
	14	7.3*** ±	0.033	4.4
	1	7.4** ±	0.202	4.5
Activia Low-Fat	7	7.5** ±	0.202	4.5
	14	7.0*** ±	0.033	4.5
	1	9.6*** ±	0.145	4.2
Actimel	7	9.2*** ±	0.145	4.2
	14	9.1*** ±	0.115	4.1
	1	8.5*** ±	0.173	4.2
Baladna with Strawberry	7	7.4** ±	0.202	4.2
	14	6.6* ±	0.173	4.2
	1	9.2*** ±	0.145	4.2
Acti-Yogho	7	7.8*** ±	0.120	4.2
	14	7.1*** ±	0.120	4.2
	1	4.6** ±	0.145	4.2
Moffedo	7	3.6*** ±	0.145	4.1
	14	3.4***±	0.033	4.1
	1	5.4** ±	0.088	4.1
Vital	7	5.3** ±	0.115	4.1
	14	4.9*** ±	0.088	4.1
	1	7.6*** ±	0.088	4.3
Activia set yogurt- Apricot and peach	7	7.0** ±	0.145	4.3
	14	6.1 ±	0.088	4.3
	1	5.9 ±	0.088	4.4
Activia set yogurt light – strawberry	7	5.2*** ±	0.088	4.4
	14	5.0 ***±	0.057	4.4
	1	6.1 ±	0.145	4.4
Activia (stirred yogurt)	7	5.8 ±	0.088	4.4
	14	5.0*** ±	0.115	4.4

*: significant difference ($p<0.05$) compared to 6.0 (log_{10}),

**: highly significant difference ($p<0.01$) compared to 6.0 (log_{10}),

***: very high significant difference ($p<0.001$) compared to 6.0 (log_{10}).

▪Activia. The counts of probiotic bacteria in 'Activia drinking yogurt' were between 8.5 and 7.3 at 1 and 14-day storage respectively, with an average of 7.8. The pH was ranged between 4.5 and 4.4 with an average of 4.4.

▪Activia low fat. The counts of probiotic bacteria in 'Activia low fat drinking yogurt' were ranged between 7.4 and 7.0 at 1 and 14-day storage respectively, with an average of 7.3. The pH was 4.5 throughout the study.

▪Actimel. The counts of probiotic bacteria in Actimel product were ranged between 9.6 and 9.1 at 1 and 14-day storage respectively, with an average 9.3. The pH was ranged between 4.1 and 4.2 with an average of 4.2.

▪Baladna with Strawberry. The counts of probiotic bacteria ranged between 8.5 and 6.6 at 1 and 14-day refrigerated storage respectively, with an average 7.5. The pH was 4.2 throughout the study.

▪Acti-yogho. The count of probiotic bacteria ranged between 9.2 and 7.1 at 1 and 14-day storage respectively, with an average of 8.0. The pH was 4.2 throughout the study.

▪Moffedo. The log of count ranged between 4.6 and 3.4 at 1 and 14-day storage respectively, with an average of 3.9. The pH was 4.1 throughout the study.

▪Vital. The count of probiotic bacteria ranged between 5.4 and 4.9 at 1 and 14-day storage respectively, with an average of 5.2. The pH was 4.1 throughout the study.

▪Activia set yogurt- Apricot and peach. The count of probiotic bacteria ranged between 7.6 and 6.1 at 1 and 14-day storage respectively, with an average of 6.9. The pH was 4.3 throughout the study.

▪Activia set yogurt light – strawberry. The count of probiotic bacteria ranged between 5.0 and 5.9 at 1 and 14-day storage respectively, with an average of 5.4. The pH was 4.4 throughout the study.

▪Activia (stirred yogurt). The count of probiotic bacteria ranged between 6.1 and 5.0 at 1 and 14-day storage respectively, with an average of 5.6. The pH was 4.4 throughout the study.

All the above products had significant difference in counts from the probiotic count of 6.0 $\log_{10}$ (the threshold) except Activia Set yogurt- Apricot- at 14 day , Activia set yoghurt light – strawberry- at 1 day , Activia (stirred yoghurt)- at 1 day and Activia (stirred yoghurt)- at 7 day. There are significant differences in counts due to the effect of time except the 'Activia low fat' and 'Actimel' products.

3.2 Organoleptic Evaluation

Table 3 shows the averages of sensory evaluation test of different probiotic dairy products marketed in Jordan. It shows that the best appearance was in the Activia with an average of 8.6, whereas the lowest appearance was in Moffedo with an average of 7.1, and the two products were significantly different in appearance. The best smell was in Actimel with an average of 8.4, whereas the lowest smell was in Baladna with strawberry with an average of 6.6, and the two products were significantly different in smell. The best consistency was in Actimel with an average of 8.3, whereas the lowest consistency was in Baladna with strawberry with an average of 6.8, and the two products were significantly different in consistency. The best taste was in Actimel with an average of 8.0, whereas the lowest taste was in Baladna with strawberry with an average of 6.5, and the two products were significantly different in taste. The best overall acceptability was in Actimel with an average of 8.1, whereas the lowest overall acceptability was in Activia set yogurt- Apricot and peach with an average of 7.0, and the two products were significantly different in overall acceptability.

Table 3. Results of the averages of sensory evaluation test of commercial probiotic dairy products (Average of 12 panelists) at 14 days of shelf life.

Products	**Appearance**[1]	**Smell**[1]	**Consistency**[1]	**Taste**[1]	**Overall Acceptability**[1]	**Average**
Activia	8.6^{a} ±0.11	7.1^{c} ±0.11	7.0^{cd} ±0.12	6.8^{fg} ±0.11	7.3^{cd} ±0.11	7.4
Activia Low-Fat	8.0^{b} ±0.13	7.4^{c} ±0.12	8.1^{a} ±0.10	7.3^{de} ±0.11	7.6^{bc} ±0.13	7.7
Actimel	8.5^{a} ±0.10	8.4^{a} ±0.11	8.3^{a} ±0.13	8.0^{a} ±0.13	8.1^{a} ±0.12	8.3
Baladna with strawberry	7.3^{ef} ±0.08	6.6^{d} ±0.11	6.8^{d} ±0.12	6.5^{g} ±0.11	7.1^{de} ±0.10	6.9
Acti-Yogho	7.5^{de} ±0.12	7.4^{c} ±0.15	7.4^{bc} ±0.13	7.3^{cde} ±0.15	7.3^{cd} ±0.15	7.4
Moffedo	7.1^{f} ±0.10	7.1^{c} ±0.16	7.1^{bcd} ±0.17	7.0^{ef} ±0.16	7.1^{de} ±0.12	7.1
Vital	7.8^{bc} ±0.10	7.2^{c} ±0.12	8.0^{a} ±0.14	7.8^{ab} ±0.13	7.8^{b} ±0.13	7.7
Activia set yogurt- Apricot and peach	7.4^{def} ±0.09	7.1^{c} ±0.14	7.0^{cd} ±0.15	7.1^{ef} ±0.14	7.0e ±0.15	7.1
Activia set yogurt light – strawberry	8.0^{b} ±0.13	7.9^{b} ±0.13	7.5^{b} ±0.12	7.6^{abc} ±0.13	7.6^{bc} ±0.15	7.7
Activia (stirred yogurt)	7.7^{cd} ±0.11	7.4^{c} ±0.12	7.2^{bcd} ±0.10	7.6^{bcd} ±0.15	7.6^{bc} ±0.13	7.5

[1]: Different letters (a, b, c,…) indicate significant difference between the same sensory property of different products based on LSD (Least significant difference).

4. Discussions

The count of probiotic bacteria were still above 6.0 log cfu/g in all samples until the end period of refrigerated storage except in four products including Moffedo, Vital and Activia set yogurt light-strawberry and Activia stirred yogurt. The highest *Bifidobacterium* count (9.6 log cfu/g) at 1st day of shelf life was in Actimel. Our results shows higher counts than Cakmakci *et al.,2010* which was (6.38 log cfu/g) for *B.bifidum*-fermented yogurt on the first day. The pH of commercial probiotic dairy products and the counts of probiotic bacteria was generally decreased during storage at 4 ^{o}C, and these results are compatible with the results of Cakmakci *et al., 2010.*

In a study of Vinderola *et al*., 2000 the cell counts of *S. thermophilus*, *L. acidophilus* and *B. bixdum* gradually decreased through the cold storage of carbonated and non-acidified fermented milks, although the counts were always higher than 10^{6} viable cells g^{-1}.

Gueimonde et al., 2004 shows that counts of Lactobacillus spp. always remained higher than 10^{5} cfu ml^{-1}, whereas the population of Bifidobacterium spp. decreased below this level in two commercial

probiotic-fermented dairy products. These results confirmed the routine counting of probiotic bacteria in marketed probiotic products, as some times the count of probiotic bacteria in these products were lower than the therapeutic dose emphasized in many literatures.

5. Conclusions

It is concluded that the counts of probiotic bacteria in fermented dairy products marketed in Jordan is not always above the therapeutic dose of 6.0 log cfu/g. This urge the governmental authorities of Jordan to establish a special standard for probiotic fermented dairy products. It is also important for dairy companies to increase their addition of the probiotic bacteria to still viable until the end of shelf life. The sensory evaluation test shows that the Jordanian people generally like to consume the probiotic fermented dairy products. This will encourage companies to produce new traditional products to enlarge the local functional food markets.

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