Pakistan Journal of Nutrition 4 (3): 170-174, 2005
ISSN 1680-5194

Effect of Dried Raisins and Apricots Extract on the Growth of *Bifidobacteria* in Cows and Goats Milk

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Abstract: The study confirms the growth of *Bifidobacteria* (*B. infantis*) in milk mixed with dried fruits (apricots and raisins) extract with no significant difference at ($p<0.05$) regardless of the milk type and the fruit extract concentration. The sensory evaluation of *bifidus milk* extracts fermented by *B. infantis* prepared from cow's and goat's milk with dried raisin and apricot extract each at 10% (v/v) concentration, showed a significant acceptability at ($P< 0.05$) for both cow's and goat's milk with apricot extract against a lower acceptability obtained for milks with raisin extract with a significant differences at ($P< 0.05$).

Key words: *B. infantis*, goat's milk, raisin, apricot

Introduction

Bifidobacteria are considered to be one of the most important genera of bacteria in terms of human health. They account for 85 to 99% of the intestinal flora in infants (Mitsuoka, 1990). All species derived from human are non-spore forming, non-motile, anaerobic, Gram-positive bacteria. In a healthy adult person *Bifidobacteria* constitute third to fourth largest group of microflora in the lower gastrointestinal tract, while coliforms, *clostridia* and *lactobacilli* normally account for less than 15% of the intestinal flora (Mitsuoka, 1984). At birth, *Bifidobacterium infantis, B. breve* and *B. longum* are dominant but are gradually replaced with *B. adolescentis. Bifidobacterium longum* persists from birth throughout life in most healthy individuals. *Bifldobacterial* counts of 10^9 to 10^{10} per gram of stool are common in adults (Mitsuoka, 1982).

Bifidobacteria had long been recognized as bacteria with probiotic, nutritive and therapeutic properties (Bezkoravainy, 2001). Since Bifidobacteria do not grow well in milk, the manufacturing of fermented milk products with *Bifidobacteria* often requires the use of an inoculum containing the final number of cells of *Bifidobacteriam* required for the products (Crittenden, 1999).

Recently there has been an increasing interest in the incorporation of the intestinal species *Lactobacillus acidophilus* and *Bifidobacterium* species into fermented milk products. These species are frequently associated with health promoting effects in human and animal intestinal tract. These probiotic effects are generally related to inhibition of pathogenic species, reducing the risk of colon cancer, increasing the immune response and decreasing concentration of cholesterol in blood plasma (Gilliland, 1990; Gurr, 1987).

Bifidobacteria are not true lactic acid bacteria in the sense of a *Lactococcus* or *pediococcus* (Hughes and Hoover, 1991). *Bifidobacteria* produce both acetic and lactic acids as primary metabolites in the molar ratio of 3:2. Glucose is degraded characteristically by the fructose 6- phosphate shunt metabolic pathway (Bezkoravainy, 2001; Holzapfel *et al.,* 1998).

Dried apricots and raisins are widely consumed and produced in Jordan and Syria and are considered to be one of the most rich sources in minerals and sugars. Furthermore, milk is one of the best sources of nutrients for child growth. The addition of fruit extracts may enhance the growth of *Bifidobacteria* by providing essential nutrients, enhancing the sensory quality of the products since the flavor of bifido culture in milk is not favorable, and providing consumers with certain nutrients especially minerals and energy.

Therefore, the objectives of the study were first to screen the ability of adding extracts of dried apricots and raisins to cow's, goat', and sheep's milk to stimulate the growth and stability of selected *Bifidobacteria* and second to investigate the acceptability of health drinks made from Milk and dried fruit (apricots and raisins) extracts fermented by *Bifidobacteria.*

Materials and Methods

Milk source and heat treatment: Whole raw Cow's milk was obtained from Alsanabel Dairy Company Ltd., and Goat's milk was from the local market (Jordan). Whole milk samples (1000 ml each) were heat treated at 93 ± 1°C for 20 min in water bath, then cooled (in fridge).

Bifidobacteria cultures: Lyophilized *Bifidobacterium infantis* ATCC 15697 was obtained from Deutsche Sammlung von Microorganismen und Zellkulturen GmbH, Braunschweig (DSMZ), Germany.

Forty-eight hours prior to the start of each experiments, cultures were revived by transfer twice into 10 ml of MRSL (MRS broth with 5% lactose), (Hughes and Hoover, 1991) and incubation at 37°C for 24 hours in an anaerobic chamber (Gaspak system; BBL, Cockeysville, MD, U.S.A).

Viability determination: *Bifidobacterium* strains *B. infantis* 15697 was cultured anaerobically at 37°C for 48 h with 0, 10, 15, and 20% (v/v) extracts of dried apricots and dried red raisin (product of Jordan). Samples containing no fruits (0%) were used as controls. All inoculated samples after fermentation were stored at 4.0 ± 1°C for 15 d. One ml of each milk samples was diluted with 9 ml of sterile 0. 1% (w/v) peptone water (Difco) and mixed uniformly with a vortex mixer (Waring Blendor, model 32BL80). Subsequent ten fold serial dilutions were prepared and viable numbers enumerated using pour plate technique. *Bifidobacteria* were enumerated in duplicate using MRSL agar (Difco, Laboratories. Detroit, MI, U.S.A). The inoculated plates were incubated anaerobically at 37°C for 48 h using N_2 gas. Cell counts were carried out on day 0, 3 6, 9 12, and 15. The colonies were counted using a colony counter (Model, EC1-video colony counter AES, Laboratoire).

Growth studies: Growth characteristics of cultures of *Bifidobacteria* in cow and goat were evaluated. Each culture was inoculated at 1% (v/v) into 100 ml of milk and was incubated at 37°C for 16 hours in the anaerobic chamber (BBL). Initial viable counts for each culture were standardized by the use of standard curve so that they were approximately the same for all cultures (=$1x10^8$ cfu/ml). Viable counts were done by serial dilution with 0.1% peptone -water and pour plating in duplicate using MRSL agar. Then samples were drawn at 0, 4, 8, 12 and 16 h from each flask and flushed with inert gas (N_2) after closing every sampling time.

Preparation of fermented and unfermented milk: Milk from goats and cows were used for preparation of fermented and unfermented according to Hughes and Hoover (1991) method.

Fermented milks were made by a 1% (v/v) inoculation of 100 ml of each milk. Flask openings (Corring Brand Milk Dilution Bottles, Screw Cap, Cat. No. 1372) were sealed with a single layer of parafilm (Parafilm, Laboratory Film, American National Can. Chicago, IL. 60631). Fermented milk was incubated for 16 hrs at 37°C in the anaerobic chamber for growth studies. Unfermented samples were prepared by inoculation of milks that had been prechilled to 4°C for 15 days. After that the sample flasks were sealed and capped as described for fermented samples. Samples were stored at 4°C immediately after inoculation. Bacterial counts was evaluated in the fermented and unfermented milks on days 0, 3, 6, 9, 12 and 15. Viable counts were determined as mentioned in the growth studies.

Dried raisins and apricots samples: One variety of dried apricots of and dried raisin product of Jordan were used(free of preservatives). The extracts were prepared with ca. 35% total solids.

Dried fruit extraction method: Dried apricots and raisin (250 g each) were soaked in 500 ml distilled water at 70° C for 1 hour, blended using Waring Blender, (model 32BL80), then strained in cheese cloth. The extracts were filled in 500ml glass bottles and sterilized at 121°C for 15 minutes in an autoclave.

Statistical analysis of experimental data: General linear model (GLM) and Fisher's least significant difference (LSD) were used to differentiate between means within and among the treatments using SAS™ (Version 8 , SAS institute (The data obtained were reduced at a significance level of 5%(% = 0.05).

Sensory evaluation: A hedonic (5 points) scale test as described by Munoz *et al*. (1992), was used to evaluate the acceptance of milks from cow and goat containing 10% raisin or apricot extract. Fourty panelists from Mu'tah University, Faculty of Agriculture conducted the sensory evaluation. Panelists were asked to evaluate aroma, taste, color and overall acceptability of the samples.

Results

***Bifidobacterium infantis* Growth in milks containing dried Apricots extract:** Dried apricot and raisin are added to different types of milk for flavoring and sweetening purposes, that may supply the added culture with suitable nutrients.

Preliminary investigations were carried out to compare growth behaviors of one species of *Bifidobacteria*. Initial viable counts for each culture were standardized by the use of standard curve so that they were approximately the same count for all (= $1x10^8$ cfu/ml). Results in Table 1 show changes in *Bifidobacterium* counts (log cfu/ml) in milks from cow and goat containing different concentrations of dried apricots extract and inoculated with 1% culture of *B. infantis*. The changes in the *Bifidobacterium* counts (log cfu/ml) in milks were not significantly different at (P< 0.05) between 0%, 10% and 20% concentrations respectively of dried apricots extract after incubation for 16 h at 37±1.0°C.

The bacterial counts (log cfu/ml) in cow's milk at all time intervals tested were ranged from 6.8 to 7.6 after incubation for 16 h at 37±1.0°C regardless of the dried apricots extract concentration against 6.3 to 6.8 of the bacterial counts (log cfu/ml) in goat's milk.

Growth in milks containing raisin extract: Results in Table 2 show changes in *Bifidobacterium* counts (log cuf/ml) in milks from cow and goat containing different concentration of raisin extract and inoculated with 1% culture of *B. infantis*. The changes in the *Bifidobacterium* counts (log cfu/ml) in milks were also not significantly different at (P<0.05) between 0, 10 and 20% concentrations.

Table 1: *Bifidobacterium* counts (log cfu/ml) in cow's and goat's milk containing different concentrations of dried apricots extract and inoculated with 1% culture of *B. infantis* when incubated at 37 ±1.0°C

Milk	Time (h)	Dried apricot concentration %		
		0	10	20
Cow	0	6.8[a1]	6.8[a]	6.4[a]
	4	7.0[a]	6.8[a]	6.4[a]
	8	6.8[a]	6.8[a]	6.8[a]
	12	7.1[a]	6.9[a]	7.1[a]
	16	7.6[a]	7.2[a]	7.6[a]
Goat	0	6.3[a]	6.8[a]	6.7[a]
	4	6.8[a]	6.8[a]	6.8[a]
	8	6.8[a]	6.8[a]	6.5[a]
	12	6.8[a]	6.8[a]	6.4[a]
	16	6.8[a]	6.8[a]	6.8[a]

[1]Means in the same rows and columns with the same letter are not significantly different at (P < 0.05)

Table 2: *Bifidobacterium* counts (log cfu/ml) in cow's and goat's milks containing different concentrations of raisin extract and inoculated with 1% culture of *B. infantis*

Milk	Time (h)	Dried raisin concentration %		
		0	10	20
Cow	0	6.0[a1]	6.0[a]	6.0[a]
	4	6.0 [a]	5.93[a]	6.0[a]
	8	6.3[a]	6.3[a]	6.4[a]
	12	6.4[a]	6.5[a]	6.3[a]
	16	6.6[a]	6.7[a]	7.1[a]
Goat	0	6.0[a]	6.8[a]	6.5[a]
	4	6.8[a]	6.8[a]	6.5[a]
	8	6.8[a]	6.8[a]	6.5[a]
	12	6.8[a]	6.8[a]	6.7[a]
	16	6.8[a]	6.8[a]	6.8[a]

[1]Means in the same rows and columns with the same letter are not significantly different at (P < 0.05).

The bacterial counts (log cfu/ml) in cow's milk raisin extract increased from 6.0 to 6.6, 6.0 to 6.7 and 6.0 to 7.1 at 0 ,10 and 20% concentrations, respectively after incubation period of 16 hr at 37 ±1.0°C.

The bacterial counts (log cfu/ml) in goat's milk raisin extract increased from 6. 0 to 6.82, and 6.5 to 6.8 at 10 and 20% raisin extract , whereas, no increase in the bacterial count at 10% remained constant throughout the period of incubation.

Viability and activity of *Bifidobacterium infantis* during refrigerated storage at 4 ±1.0°C

Milks containing dried apricots extract: Results in Table 3 show changes in *Bifidobacterium* counts (log cfu/ml) in milks from cow and goat containing different concentration of dried apricots extract and inoculated with 1% culture of *B. infantis*. The changes in the *Bifidobacterium* counts (log cfu/ml) in milk from cow containing 0, 10 and 20% dried apricots extract were significantly different after 15 days of storage at 4 ±1.0°C. The changes in the *Bifibacterium* counts (log cfu/ml) between 0 and 10% concentration of dried apricots extract were also not significantly different.

The *Bifidobacterium* count (log cfu/ml) slightly decreased from 7.0 to 6.4, and from 6.8 to 6.4 at 10 and 20% concentrations, respectively but it slightly increased from 6.5 to 6.8 at 0% concentration of dried apricots extract at 15th day of storage at 4±1.0°C.

Table 3: Bifidobacterium counts (log cfu/ml) in cow's and goat's milk containing different concentrations of dried apricots extract and inoculated with 1% culture of *B. infantis* when incubated at 4 ±1.0ºC for 15 days

Milk	Time (day)	Dried apricots concentration %		
		0	10	20
Cow	0	6.5[a1]	6.8[a]	7.0[b]
	3	6.7[a]	6.5[a]	6.7[b]
	6	6.5[a]	6.8[a]	6.5[b]
	9	6.4[a]	6.1[a]	6.5[b]
	12	6.7[a]	6.7[a]	6.5[b]
	15	6.8[a]	6.4[a]	6.4[b]
Goat	0	6.3[a]	6.8[a]	6.8[ab]
	3	6.4[a]	6.80[a]	5.6[ab]
	6	6.4[a]	6.4[a]	5.8[ab]
	9	6.2[a]	6.3[a]	6.3[ab]
	12	6.5[a]	6.6[a]	6.4[ab]
	15	6.5[a]	6.4[a]	6.3[ab]

[1]Means in the same rows and columns with the same letter are not significantly different at (P < 0.05).

Table 4 : *Bifidobacterium* counts (log cfu/ml)in cow's and goat's milk containing different concentrations of raisin extract and inoculated with 1% culture of *B. infantis* when incubated at 4± 1.0ºC for 15 days

Milk	Time (day)	Raisin concentration %		
		0	10	20
Cow	0	6.0[a1]	6.1[a]	6.0[a]
	3	6.6[a]	6.3[a]	6.8[a]
	6	6.1[a]	6.2[a]	6.8[a]
	9	6.4[a]	6.6[a]	6.4[a]
	12	6.5[a]	6.3[a]	6.4[a]
	15	6.5[a]	6.5[a]	6.3[a]
Goat	0	6.3[a]	6.5[a]	6.8[a]
	3	6.8[a]	6.5[a]	6.8[a]
	6	6.8[a]	6.8[a]	6.8[a]
	9	6.8[a]	6.8[a]	6.8[a]
	12	6.8[a]	6.8[a]	6.5[a]
	15	6.8[a]	7.0[ab]	6.3[a]

[1]Means in the same rows and columns with the same letter are not significantly different at (P < 0.05)

Table 5: Sensory evaluation[1] of cow's and goat's milk containing 10% of raisin extract

Milk	Fruit Extracts	Aroma	Taste	Color	Overall acceptability
Cow	None	4.0[a]	3.4[a]	3.4[a]	3.6[a]
	Raisins	3.1[2b]	3.0[b]	3.3[a]	3.6[a]
	Apricots	4.2[a]	4.0[a]	3.3[a]	4.0[a]
Goat	None	3.5[a]	3.4[a]	3.5[a]	3.4[a]
	Raisins	2.6[b]	2.8[b]	2.9[a]	2.6[b]
	Apricots	3.6[a]	4.0[a]	3.0[a]	3.4[a]

[1]A hedonic scale presented in 5 categories (1 ; dislike very much, 2; dislike, 3; neither dislike nor like, 4; like and 5; like very much).
[2]Means (n = 40) with different letters within a column are significantly different at ($p<0.05$).

Table 3 shows that changes in *Bifidobacterium* counts (log cfu/ml) in goat's milk containing different concentrations of dried apricots extract and inoculated with 1% culture of *B. infantis* were significant at ($P< 0.05$) throughout the period of storage, the counts (log cfu/ml) decreased from 6.8 to 6.4 and 6.8 to 6.3 at 10%, and 20% concentrations, but the count (log cfu/ml) increased from 6.3 to 6.5 at 0% concentration of dried apricots extract after 15 days of storage at 4 ±1.0ºC .

Milks containing raisin extract: Results in Table 4 show changes in *Bifidobacterium* counts (log cfu/ml) in cow's, and goat's milk containing different concentrations of raisin extract and inoculated with 1% culture of *B. infantis*. The changes in the *Bifidobacterium* counts (log cuf/ml) in milk from cow containing 0, 10 and 20% raisin extract weren't significantly different at $p<0.05$ level at refrigerated storage for 15 days at 4±1.0ºC.

The *Bifidobacterium* count (log cfu/ml)in milk from cow at all time intervals tested, slightly increased from 6.0 to 6.5, 6.8 to 7.0 at 0%, 10% concentration, whereas, decreased from 6.8 to 6.3 at 20% concentrations, respectively, of raisin extract after 15 days of refrigerated storage at 4±1.0ºC.

Table 4 shows also that changes in *Bifidobacterium* counts (log cfu/ml) in goat's milk containing different concentrations of raisin extract and inoculated with 1% culture of *B. infantis* were not significantly different at all concentration.

Sensory evaluation: The result of the sensory evaluation presented in Table 5 shows that the cow's milk drinks were evaluated as the most acceptable drink as they scored 3.6 and 4.0 for milk with raisin and apricot extract, respectively. This means that milk with apricot extract is more preferable and its acceptance lies between “neither like nor dislike” and “like” in other words they were very close to the like rating and the drink with apricot extract is more favourable by panels than milk or milk with raisins extract.

The goats milk with raisin extract had the lowest overall acceptability, the rating was 2.6 against 3.4 for milk and milk with apricot. It is also, clear from the results obtained that the aroma of the cows milk with raisins for both cow’s and goats milk were the lowest as they score 3.1 and 2.6 for cow’s and goats, respectively. Furthermore, the aroma, taste and colour of milk drink mixed with raisins extract were the least acceptable as they scored 2.6, 2.8 and 2.9 for aroma taste and color, respectively.

Discussion

The production of bifidobacteria fermented milk is not easy compared to yoghurt fermentation. In addition, the taste and aroma of the products are not favorable (Rasic, 1983; Roy *et al.,* 1990).

The use of dried fruit extracts with milk is a continuation of many previous researches to enhance the growth and viability of bifidobacteria (Klaver *et al.,* 1993; Roy *et al.,* 1990; Shin *et al.,* 2001). Raisins and dried apricots were selected in this study for their popularity in the middle east countries and for their high carbohydrates and mineral contents. Moreover, these products are available all over the year and they are one of the main food items always found on the food banquets during the holly month of Ramadan.

The results of the study of growth of *Bifidobacterium* during refrigerated storage, presented in the Table 3 and 4 generally revealed no significant increase in of bacterial count.

Growth promotion, enhancement of activity and retention of viability were greatest when *Biftdobaacrium* were grown in the presence of fructooligosaccharide (FOS), followed in a descending order by galactooligosacchride (GOS) and inulin. The effects of oligosaccharides and inulin increased with increasing carbohydrate concentration (Shin *et al*., 2000).

The loss in viability of *Bifidobacteria* occurs in fermented milks could be due to acid formation and presence of oxygen (Shah, 2000). Bifidobactria also could grow well in milk inoculated with cultures prepared in a synthetic medium.

This study agree with Shah *et al*. (1995) who studied the survival of *Bifidobacterium bifidum* in five brands of commercial yogurt during refrigerated storage and found that the number of viable counts *B. bifidum* steadily declined in all the products during refrigerated storage. The loss of viability was attributed to the decrease in pH values during refrigerated storage.

Loss of viability of *Bifidobacteria* is typically more pronounced in fermented milk than in unfermented milk due to acid injury to the organism (Dave and Shah,

1997). Lankaputhra *et al.* (1996) observed that viability of *Bifidobacteria* strains such as *Bifidobacterium infantis* in 12% skim milk at pH 4.3 was decreased by 30% after 12 d of storage at 4°C. After 24 d the same temperature, the counts decreased by more than 82%. Medina and Jordan (1994) observed a 93% reduction in bifidobacterial counts of fermented milk produced in Spain at 7°C .

On the contrary, doubling of bacterial count of *B*. *Bifidum* cultured in skim milk mixed with honey, fructose or glucose was found by Ustunol and Gandi (2001) after 222 minutes of incubation.

Since most of the cfu numbers in all preparations are 10^6 cfu/ml or higher, it is concluded, according to the Adhikari *et al.* (2003), that all preparations are adequate, provided a minimum of 100 ml is consumed daily.

The results of the sensory quality presented in table Table 4 showed that cow's milk mixed with both fruit extracts (Raisins and apricots) have a moderate acceptance for and the least acceptance for goat's milk mixed with raisins extract .

These results are in agreement with Al-saleh results (2001) that indicated a low sensory acceptability of Bifidus milk. The sensory acceptability of Bifidus milk preparation could be improved by combining Bifidus with acidophillus fermentations (Gomes and Malcata, 1999) or by two steps fermentation (Adhikari *et al*., 2003), since this will result in increasing the sourness of the product.

Conclusions: The inoculation of milk at levels $\geq 10^6$ cfu/ml is adequate for the production of Bifidus milk with a probiotic effect. Also, a cold storage of inoculated milks with Bifidobacteria showed of a bacterial growth which enhance the formulation of fruit flavoured of bifidus milk by inoculation.

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