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PJBS

ISSN 1028-8880

Pakistan Journal of Biological Sciences

ANSInet
Asian Network for Scientific Information
308 Lasani Town, Sargodha Road, Faisalabad - Pakistan

Pakistan Journal of Biological Sciences 9 (1): 128-132, 2006
ISSN 1028-8880

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

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Abstract: The growth of *Bifidobacteria* (*B. infantis)* in milks mixed with dried fruit extracts (Apricot) was insignificantly affected ($p>0.05$) by either of the milk type and/ or the fruit extract concentration. Moreover, the sensory evaluation of *bifidus* milk extracts fermented by *B. infantis* prepared from cow's and sheep's milk with dried apricot extract each at 10% (v/v) concentration, showed a significant acceptability at $p<0.05$ for both cow's and sheep's milk with apricot extract.

Key words: *B. infantis,* sheep's milk, 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[1]. 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[2]. 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} g^{-1} of stool are common in adults[3].

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[4].

Recently there has been an increasing interest in the incorporation of the intestinal species of *Lactobacillus acidophilus* and *Bifidobacterium* 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[5,6].

Bifidobacteria are not true lactic acid bacteria in the sense of a *Lactococcus* or *Pediococcus*[7]. *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[8,9].

Dried apricot is widely consumed and produced in Jordan and syria and is considered to be one of the most rich sources in minerals and sugars. Further, 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 bacteria* culture in milk is not favorable. However, it is 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 to cow's and sheeps milk to stimulate the growth of *Bifidobacteria* and second to investigate the acceptability of health drinks made from milk and dried fruit (apricots) extracts fermented by *Bifidobacteria.*

MATERIALS AND METHODS

Milk source and heat treatment: Whole raw Cow's and sheep's milk was obtained from Alsanabel Dairy Company Ltd. in march 2004. Whole milk samples (1000 mL each) were heat treated at 93±1°C for 20 min in water bath, then cooled (in fridge)[10,11].

***Bifidobacteria* cultures:** Lyophilized *Bifidobacterium infantis* ATCC 15697 was obtained from Deutsche SammLung von Microorganisme and Zellkulturen GmbH, Braunschweig (DSMZ), Germany.

Forty-eight hours prior each experiment, cultures were transfer twice into 10 mL of MRSL (MRS broth with 5% lactose)[7] and incubated at 37°C for 24 h in an anaerobic chamber (Gaspak system; BBL, Cockeysville, MD, USA).

Viability determination: *Bifidobacterium* strains *B. infantis* 15697 was cultured anaerobically at 37°C for 48 h with 0, 10 and 20% (v/v) extracts of dried apricots (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 12 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. Subsequent ten fold serial dilutions were prepared and viable numbers enumerated using pour plate technique. *Bifidobacteria* were enumerated in duplicate using MRSL agar. 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 and 12. The colonies were counted using a colony counter (Model, EC colony counter AES, Laboratoire).

Growth studies: Growth characteristics of cultures of *Bifidobacteria* in cow and sheep milks were evaluated. Each culture was inoculated at 1% (v/v) into 100 mL of milk and was incubated at 37°C for 16 h 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^{-1}). 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 sheep's and cow's were used for preparation of fermented and unfermented according to Hughes and Hoover[7] method. Fermented milks were made by a 1% (v/v) inoculation of 100 mL of each milk. Flask openings were sealed with a single layer of parafilm. Fermented milk was incubated for 16 h 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 12 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 and 12. Viable counts were determined as mentioned in the growth studies.

Dried apricots samples: One variety of dried apricots product of Jordan were used (free of preservatives). The extracts were prepared with ca. 30% total solids.

Dried fruit extraction method: Dried apricots (250 g each) were soaked in 500 mL distilled water at 70°C for 1 h, blended using Waring Blender (model 800 S, USA), then strained in cheese cloth. The extracts were filled in 500 mL glass bottles and sterilized at 121°C for 15 min in an autoclave[10].

Statistical analysis: 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% (α 0.05).

Sensory evaluation: A hedonic (5 points) scale test as described by Munoz *et al.*[12] was used to evaluate the acceptance of milks from cow and sheep containing 10% apricot extract. The researchers (Panelists) were asked to evaluate aroma, taste, color and overall acceptability of the samples.

RESULTS

Growth *of B. infantis* in milk containing dried apricots extract: Dried apricot was 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 (B. infantis)*. 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^{-1}). Results in Table 1 show changes in *Bifidobacterium* counts (log cfu/mL) in milks from cow and sheep containing different concentrations of dried apricots extract and inoculated with 1% culture of *B. infantis*. The changes in the *Bifidobacterium* counts (log cfu mL^{-1}) 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.

Table 1: *Bifidobacterium* counts (log cfu mL^{-1}) in cow's and sheep's milk containing different concentrations of dried apricots extract and inoculated with 1% culture of *B. infantis* when incubated at 37±1.0°C for 16 h[1]

		Dried apricots concentration %		
Milk	Time (h)	0	10	20
Cow's	0	6.8[a]	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]
Sheep's	0	5.7[a]	6.2[a]	6.3[a]
	4	6.4[a]	6.2[a]	6.8[a]
	8	6.4[a]	6.3[a]	6.5[a]
	12	6.3[a]	6.2[a]	6.4[a]
	16	6.5[a]	6.4[a]	7.0[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^{-1}) in cow's and sheep's milk containing different concentrations of dried apricots extract and inoculated with 1% culture of *B. infantis* when incubated at 4±1.0°C for 12 days[1]

		Dried apricots concentration %		
Milk	Time (day)	0	10	20
Cow's	0	6.5[a]	6.8[a]	7.0[a]
	3	6.7[a]	6.5[a]	6.7[a]
	6	6.5[a]	6.8[a]	6.5[a]
	9	6.4[a]	6.3[a]	6.5[a]
	12	6.8[a]	6.7[a]	6.5[a]
Sheep's	0	6.3[a]	6.8[a]	6.8[a]
	3	6.4[a]	6.8[a]	5.6[a]
	6	6.4[a]	6.4[a]	5.8[a]
	9	6.2[a]	6.3[a]	6.4[a]
	12	6.5[a]	6.4[a]	6.4[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^{-1}) in cow's milk at all time intervals tested were ranged from 6.4 to 7.6 after incubation for 16 h at 37±1.0°C regardless of the dried apricots extract concentration against 5.7 to 7.0 of the bacterial counts (log cfu mL^{-1}) in sheep's milk.

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

Milks containing dried apricots extract: Results in Table 2 show changes in *Bifidobacterium* counts in milks from cow and sheep containing different concentration of dried apricots extract and inoculated with 1% culture of *B. infantis*. The changes in the *Bifidobacterium* counts in milk from cow containing 0, 10 and 20% dried apricots extract were insignificantly different after 12 days of storage at 4±1.0°C. The changes in the *Bifibacterium* counts (log cfu mL^{-1}) between 0 and 10% concentrations of dried apricots extract were also not significantly different.

The *Bifidobacterium* count slightly decreased from 6.8 to 6.4 and from 7.0 to 6.4 (log cfu mL^{-1}) at 10 and 20% concentrations, respectively, but it was slightly increased from 6.5 to 6.8 (log cfu mL^{-1}) at 0% concentration of dried apricots extract at 12 th day of storage at 4±1.0°C in cow's milk.

Table 2 shows that changes in *Bifidobacterium* counts in sheep's milk containing different concentrations of dried apricots extract and inoculated with 1% culture of *B. infantis* were insignificant at $p<0.05$ throughout the period of storage of the same. The counts were decreased from 6.8 to 6.4 (log cfu mL^{-1}) at 10 and 20% concentrations. On the contrary, the count showed a small insignificant increase from 6.3 to 6.5 (log cfu mL^{-1}) at 0% concentration of dried apricots extract after 12 days of storage at 4±1.0°C for sheeps milk.

Sensory evaluation: The result of the sensory evaluation presented in Table 3 shows that both cows and sheep's milk mixed with apricots extract (10%) were evaluated as the most acceptable drink as they scored 4.0 for milk mixed with apricot extract.

Table 3: Sensory evaluation[1] of cow's and sheep's milk containing 10% of apricots extract

Milk	Fruit extracts	Aroma[2]	Taste	Color	Overall acceptability
Cow's	None	3.8[a]	3.4[a]	3.4[a]	3.6[a]
	Apricots	4.0[a]	4.0[a]	3.0[a]	4.0[a]
Sheep's	None	3.5[a]	3.4[a]	3.4[a]	3.4[a]
	Apricots	3.6[a]	4.0[a]	3.1[a]	4.0[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 litters within a column are significantly different at ($p<0.05$)

This means that milk with apricots 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 which is more favourable by researchers (panelists).

The sheep's milk with appricots extract had the same overall acceptability as cow's milk. The rating was 4.0 milk with apricot. It is also, clear from the results obtained that the aroma of the cows milk with apricot extract or without had a lower preferences as they score 3.6 and 3.5 for sheeps milk with and without apricot extract, respectively. Furthermore, the colour of milk drink mixed with apricot extract were the least acceptable as they scored 3.4 for the plain milk, compared with 3.0 and 3.1 for color of cows and sheep's milk mixed with Apricots extract.

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[13,14].

The use of dried fruit extracts with milk is a continuation of many previous researches to enhance the growth and viability of *Bifidobacteria*[14-16]. Dried apricots were selected in this study for their popularity in the middle east countries and for their high carbohydrates and mineral contents. Moreover, this 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 2 generally revealed no significant increase in bacterial count.

Growth promotion, enhancement of activity and retention of viability were greatest when *Bifidobaacrium* were grown in the presence of fructooligosaccharide (FOS), followed in a descending order by galactooligosacchride (GOS) and inuIin. The effects of oligosaccharides and inulin increased with increasing carbohydrate concentration[16].

The loss in viability of *Bifidobacteria* occurs in fermented milks could be due to acid formation and presence of oxygen[17]. *Bifidobactria* also could grow well in milk inoculated with cultures prepared in a synthetic medium.

This study agree with Shah *et al.*[18] 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 the unfermented milk due to acid injury to the organism[19]. Lankaputhra *et al.*[20] observed that viability of *Bifidobacteria* strains such as *Bifidobacterium infantis* in 12% skim milk at pH 4.3 was decreased by 30% after 12 day of storage at 4°C. After 24 day at the same temperature, the counts decreased by more than 82%. Medina and Jordano[21] 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 Ustinol and Gandhi[22] after 222 min of incubation.

Since most of the cfu numbers in all preparations were 10^6 cfu mL^{-1} or higher, it is concluded, according to the Adhikari *et al.*[23], that all preparations are adequate and provided a minimum number of *Bifidobacterium* when a 100 mL of *bifidus* milk is consumed daily.

The results of the sensory quality presented in Table 3 showed that cow's and sheep's milk mixed with apricots extracts had a good acceptance compared with plaine milk. The sensory acceptability of *bifidus* milk preparation could be improved by combining *bifidus* with acidophillus fermentations[24] or by two steps fermentation[23], since this will result in increasing the sourness of the product.

It may be concluded that the production of flavoured *bifidus* milk with a probiotic effect could be produced by inoculation of milk with fruit extracts, in particular dried apricot extracts. Also, a cold storage of inoculated milks with *Bifidobacteria* showed that bacterial growth enhance with the formulation of fruit flavoured of *bifidus* milk by inoculation.

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