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Effect of Dried Dates Extract on the Growth and Viability of *Bifidobacteria* in Different Milk Types

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Abstract: The objectives of the study were to screen the ability of two of dates (hilwa and sukary) extracts to stimulate the growth of two selected *strains of Bifdobacteria* (*B. infantis, B. angulatum*) inoculated in three milk types (cow, goats and camel), to investigate their effects on the stability of the bacteria during cooled storage and to study the feasibility of production of acceptable healthy drinks based on milk. Dates extract were prepared by hot soaking of dried fruits at 70°C, then sterilized at 120°C and finally mixed with milk types at three concentration levels (10, 15, 20% v/v). The growth study was conducted by inoculation of milk types and their pasteurized products (90°C / 20 min) with *Bifidobacterium infantis* or *B. angulatum* to contain 10^6 cfu/ml and incubated at 37°C for 16 h. The bacterial counts were conducted at the beginning and every four hours for a period of 16 hr. The inoculated milk products were also stored at 4°C for 15 days during which the viability of the bacteria, hydrogen ion concentration was measured and the coagulation was monitored every three days for the same periods. No significant differences were found in the growth of the two starter cultures in most treatments, regardless of the type of milk and dates extract as well as concentration and incubation time. The pH of the control milk samples as well as that of the different milk preparations decreased gradually during incubation and refrigerated storage at 4°C for 15 days. This indicated activity of the bacteria and/or their enzyme systems. The sensory evaluation of six selected bifidus milk prepared from cow's, goat's and camel's milk and hilwa dates extracts at 10% (v/v) level, revealed a moderate acceptability with no significant differences from cow's milk with hilwa dates extract and camel's milk with hilwa dates extract preparations, the least significant acceptance at ($P< 0.05$) was observed for goat's milk preparations.

Key words: *Bifdobacteria*, hilwa, sukary, camel, goat

Introduction

Bifodobacteria were isolated from various animals (Scardovi, 1986). All species derived from human are non-spore forming, non-motile, anaerobic, Gram-positive bacteria. *Bifidobacteria* had long been recognized as bacteria with probiotic, nutritive and therapeutic properties (Bezkoravainy, 2001; Holzapfel *et al*., 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). In recent years, 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).

In selecting strains to produce these products, criteria include not only properties related to the intestinal effects, but also multiplication in milk and survival during storage in acidic milk. Growth of *Bifidobacterium* in the intestine is often related to the presence of specific growth factors (Modler *et al*., 1990). *Bifidobacteria* have a number of beneficial effects on host health in both infants and adults. The most important of these include inhibition or displacement of undesirable microorganisms, elimination of procarcinogens, immunomodulation and vitamin production (Gibson and Wang, 1994; Modler *et al*., 1990).

Bifidobacteria are now big business in Japan and Europe where bifid-amended foods and beverages are one of the fastest-growing segments of culture-products. *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).

Dates are widely consumed and produced in the Kingdom of Saudi Arabia 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. Milk and dates are major food sources of Arabs in all desert region and they are often eaten simultaneously or in different combinations. 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. The objectives of the study were to screen the ability of adding extracts of date to cow's, goat's, and camel's milk in stimulating the growth and stability of selected *Bifidobacteria* and to investigate feasibility of production of acceptable health drinks based on milk and date extracts fermented by *Bifidobacteria*, and to evaluate sensory acceptability of products as health drinks.

Materials and Methods

Raw milk: Raw Cow's milk was obtained from Almarai Company Ltd., Saudi Arabia. Camel's milk was obtained from Alwatania and Goat's milk was obtained from local farm in Gurayat province in Saudi Arabia.

Heat treatment of milk: Whole milk samples (2000 ml each) were heat treated at 90°C for 20 min in water bath, then cooled (in a water bath mixed with ice).

Sources and maintenance of cultures (enumeration of *Bifidobacteria* strains)

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 experiment, cultures were revived by transfer twice into 10 ml of MRSL (MRS broth with 5% lactose), (Hughes and Hoover, 1995) and incubation at 37°C for 24 hours in an anaerobic chamber (Gaspak system; BBL, Cockeysville, MD, U.S.A).

Determination of viability during refrigerated storage: *Bifidobacterium* strains *B. infantis* 15697 was cultured anaerobically at 37°C for 48 h with 0, 10, 15, and 20% (v/v) extracts of suckary dates and hilwa dates product of Saudi Arabia and 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 Gas Paks. 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, goat and camel milks 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).

Samples were drawn from each flask at, 0, 4, 8, 12 and 16 h. and gas generating envelope was replaced at each interval.

Viable counts were done by serial dilution with 0.1% peptone-water and pour plating in duplicate using MRSL agar. The pH of samples and coagulation were determined at each interval.

Preparation of fermented and unfermented milk: The procedure of Hughes and Hoover (1995) was used to prepare fermented and unfermented whole milks from cows, goats and camels, respectively.

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, coagulation and pH were 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. The pH of samples were recorded prior to dilution.

Dried date samples: Two varieties of commercial dates product of Saudi Arabia (hilwa and suckary dates) were used. The extracts were prepared with ca. 50% total solids.

Dried dates extraction method: Samples of dates without stones (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.

Measurement of pH: The pH of the fermented and unfermented samples was measured using a pH meter (Model, Jenway 3410 Electrochemistry analyzer),

calibrated with fresh pH 4.0 and 7.0 standard buffers.

Statistical analysis of experimental data: Analyses of variance (ANOVA) procedure of SAS institute, Inc. (2000) software, general linear model (GLM) and Fisher's least significant difference (LSD) were used to differentiate between means within and among the treatments. All analysis were preformed using procedures for the general linear model(PROC GLM) of SAS ™ (Version 8 e SAS institute. (All the experiments were repeated in duplicates on different days. The data obtained were analyzed at 5% level of significance.

Sensory evaluation: A hedonic (5 points) scale test as described by Linda *et al*. (1991), was used to evaluate the acceptance of milks from cow, goat, and camel containing 10% hilwa dates extract. Twenty panelists from Quality Control Lab of ministry of commerce and industry in Haditha - Kingdom of Saudi Arabia conducted the sensory evaluation. Panelists were asked to evaluate aroma, taste, color and overall acceptability of the samples.

Results

Growth of *Bifidobacterium infantis* in flavored fruit milks containing Date extract and incubated 37°C for 16 hours

Hilwa dates extract: Results in Table 1 show changes in *Bifidobacterium* counts (log cfu/ml) in milks from cow, goat and camel containing different concentration of hilwa dates 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 ,15 and 20% concentrations, respectively of hilwa dates extract after incubation for 16 h at 37°C.

The bacterial counts (log cfu/ml) in cow's milk increased from 5.63 to 7.25, 6.53 to 7.26 , 6.82 to 7.14 and 6.79 to 7.10 at 0% ,10%, 15% and 20% concentrations, respectively of hilwa dates extract after incubation for 16 h at 37°C.

The bacterial counts (log cfu/ml) in goat's milk increased from 6.80 to 6.86, 6.81 to 6.86, 6.81 to 6.86, 6.82 to 6.86 at all concentrations of hilwa dates extract throughout the incubation for 16 h at 37°C.

In camel's milk containing different concentration of hilwa dates extract and inoculated with 1% culture of *B. infantis* the changes in *Bifidobacterium* counts (log cfu/ml) were also not significantly different at (P< 0.05) throughout the period of the incubation for 16 h at 37°C. The counts increased from 6.76 to 6.78 and 6.72 to 6.84, 6.79 to 6.84, 6.79 to 6.82 at 0%, 10% , 15% and 20% concentrations, respectively at the end of incubation for 16 h at 37°C.

The changes in pH of cow's, goat's and camel's milk containing different concentrations of hilwa dates extract and inoculated with 1% culture of *B. infantis* are shown in Table 2. The pH at all time intervals tested, were not significantly different at (P< 0.05) between 0, 10 ,15 and 20% concentrations, respectively of hilwa dates extract after incubation for 16 h at 37°C.

The pH of cow's milk decreased and ranged from 6.69 to 6.46, 6.68 to 6.59, 6.697 to 6.57 and 6.67 to 6.56 at 0, 10 ,15 and 20% concentrations, respectively, of hilwa dates extract after incubation for 16 h at 37°C.

The pH in goat's milk containing different concentrations of hilwa dates extract and inoculated with 1% culture of *B. infantis* decreased from 6.45 to 6.38, 6.43 to 5.52 , 6.42 to 5.95, and from 6.41 to 5.55 at 0, 10, 15 and 20% concentrations, respectively, of hilwa dates extract after incubation for 16 h at 37°C.

The bacterial growth (log cfu/ml) in goat's milk increased from 6.77 to 6.86, 6.76 to 6.84, 6.74 to 6.83 and from 6.79 to 6.83 at all concentrations of suckary dates extract throughout the incubation for 16 h at 37°C.

In camel's milk containing different concentration of suckary dates a extract and inoculated with 1% culture of *B. infantis* the changes in *Bifidobacterium* counts (log cfu/ml), the counts increased from 6.53 to 6.48 , 6.55 to 6. 73, 6.49 to 6.84 and from 6.53 to 6.69 after 12 hr and then decreased to 6.48 at 0%, 10% , 15% and 20% concentrations, respectively, at the end of incubation for 16 h at 37°C.

In camel's milk containing different concentrations of hilwa dates extract inoculated with 1% culture of *B. infantis*, the pH of each concentration decreased from 6.61 to 6.05, 6.61 to 5.54, 6.62 to 5.48 and from 6.60 to 5.63 during incubation for 16 h at 37°C.

Suckary dates extract: Results in Table 3 show changes in *Bifidobacterium* counts (log cfu/ml) in milks from cow, goat and camel containing different concentration of hilwa dates extract and inoculated with 1% culture of *B. infantis*. The changes in the *Bifidobacterium* counts (log cfu/ml) in milks did not significantly differ at (P<0.05) between 0, 10 ,15 and 20% concentrations, respectively of suckary dates extract after incubation for 16 h at 37°C.

The bacterial counts (log cfu/ml) in milk from cow increased from 7.13 to 7.37, 7.18 to 7.29, 7.12 to 7.18 and 7.24 to 7.48 at 0, 10,15 and 20% concentrations, respectively of suckary dates extract after incubation for 16 hr at 37°C.

The bacterial growth (log cfu/ml) in goat's milk increased from 6.77 to 6.82, 6.76 to 6.84, 6.74 to 6.83 and from 6.79 to 6.83 at all concentrations of suckary dates extract through the incubation for 16hr at 37°C.

In camel's milk containing different concentrations of suckary dates extract and inoculated with 1% culture of *B. infantis*, the changes in *Bifidobacterium* counts (log cfu/ ml) increased from 6.53 to 6.48, 6.55 to 6.73, 6.49 to 6.84 and from 6.53 to 6.48 at 12 hr, at 0, 10, 15 and 20% concentrations, respectively, at the end of incubation for 16 hr at 37°C.

Table 1: Changes in the *Bifidobacterium* counts (log cfu/ml) in cow's , goat's and camel's milk containing different concentrations of hilwa dates extract and inoculated with 1% culture of *B. infantis* when incubated at 37°C

Milk	Time (h)	Hilwa dates concentration %			
		0	10	15	20
Cow	0	6.63[a1]	6.53[a]	6.82[a]	6.79[a]
	4	6.51[a]	6.79[a]	6.84[a]	6.81[a]
	8	6.68[a]	6.61[a]	7.26[a]	6.71[a]
	12	7.04[a]	6.91[a]	6.76[a]	7.05[a]
	16	7.26[a]	7.26[a]	7.14[a]	7.10[a]
Goat	0	6.80[a]	6.81[a]	6.81[a]	6.82[a]
	4	6.80[a]	6.83[a]	6.86[a]	6.83[a]
	8	6.84[a]	6.86[a]	6.86[a]	6.86[a]
	12	6.86[a]	6.86[a]	6.86[a]	6.86[a]
	16	6.86[a]	6.86[a]	6.85[a]	6.86[a]
Camel	0	6.76[a]	6.72[a]	6.79[a]	6.79[a]
	4	6.81[a]	6.84[a]	6.81[a]	6.85[a]
	8	6.84[a]	6.84[a]	6.85[a]	6.88[a]
	12	6.85[a]	6.85[a]	6.93[a]	6.86[a]
	16	6.78[a]	6.84[a]	6.84[a]	6.82[a]

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

Table 2: Changes in the pH of cow's, goat's and camel's milk containing different concentrations of hilwa dates extract and inoculated with 1% culture of *Bifidobacterium infantis* when incubated at 37°C

Milk	Time (h)	Hilwa dates concentration %			
		0	10	15	20
Cow	0	6.69[a1]	6.68[a]	6.69[a]	6.67[a]
	4	6.64[a]	6.64[a]	6.65[a]	6.66[a]
	8	6.6[a]	6.62[a]	6.6[a]	6.62[a]
	12	6.6[a]	6.57[a]	6.54[a]	6.55[a]
	16	6.46[a]	6.59[a]	6.57[a]	6.56[a]
Goat	0	6.45[a]	6.43[a]	6.42[a]	6.41[a]
	4	6.38[a]	6.33[a]	6.32[a]	6.31[a]
	8	6.37[a]	6.33[a]	6.31[a]	6.31[a]
	12	6.42[a]	6.15[a]	6.19[a]	6.26[a]
	16	6.38[a]	5.52[a]	5.95[a]	5.55[a]
Camel	0	6.61[a]	6.61[a]	6.62[a]	6.60[a]
	4	6.29[a]	6.25[a]	6.27[a]	6.31[a]
	8	6.15[a]	6.27[a]	6.28[a]	6.29[a]
	12	6.09[a]	5.79[a]	5.89[a]	6.13[a]
	16	6.05[a]	5.54[a]	5.48[a]	5.63[a]

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

The changes in pH of cow's, goat's, and camel's milk containing different concentrations of suckary dates extract inoculated with 1% culture of *B. infantis* are shown in Table 4. The pH at all time intervals tested, were not significantly different at (P<0.05) between 0, 10,15 and 20% concentrations, respectively of suckary dates extract after incubation for 16 h at 37°C.

The pH of cow's milk decreased from 6.92 to 5.24, 6.90 to 5.41, 6.91 to 5.92 and from 6. 86 to 5.98 at 0, 10, 15 and 20% concentrations, respectively, of hilwa dates extract after incubation for 16 h at 37°C.

The pH of goat's milk containing different concentrations of hilwa dates extract and inoculated with 1% culture of *B. infantis* decreased from 6.79 to 6.16, 6.75 to 5.86, and 6.72 to 5.81, and from 6.70 to 5.80 at 0, 10, 15 and 20% concentrations, respectively, of hilwa dates extract after incubation for 16 h at 37°C.

In camel's milk containing different concentrations of suckary dates extract inoculated with 1% culture of *B. infantis*, the pH decreased from 6.73 to 6.17, 6.73 to 6.02, 6.75 to 6.05 and from 6.76 to 6.29 at 0, 10, 15, and 20% concentrations, respectively, of hilwa dates extract after incubation for 16 h at 37°C.

Sensory evaluation: The results in Tables 5 show that the cow's milk drinks with hilwa dates extract obtained the highest overall acceptability of 3.5 and 3.4 respectively; this means that their acceptance is between "neither dislike nor like" and "like" in other words they were of moderate acceptance.

Discussion

Dates was selected in this study for their high carbohydrates, dietary fibers and mineral contents. Furthermore, they are available all over the year and are stable and relatively of low cost per unit extractable dry matter. In addition, dates are the most popular and favorable food items during the holly month of Ramadan. The results of the study of growth, pH and viability and activity of *Bifidobacterium* during refrigerated storage, presented in the tables 1 to 4 generally revealed no significant growth of bacterial numbers, but considerable and sometimes significant decrease of the pH of the milks. The decrease of the pH with incubation time indicates the activity of the bacteria. A gradual and variable pH drop was recorded almost in all treatments due to incubation time. Generally, the drop was higher in camel's and goat's milk preparations than in cow's milk preparations. On the other hand, the drop was higher in enriched milk than in the pure milk (control). One can also observe a general trend of a greater decrease in milk preparations with 10 and 15% extracts additions than those with 20% extract additions, that indicates the existence of a concentration optimum for the bacterial activity. However, no clear relationship was observed between pH drop and coagulation of the milk preparation. Most coagulations occurred after 12 or 16 h of incubation or 12 days of refrigerated storage at pH values higher than the isoelectric point of casein. This

Table 3: Changes in the *Bifidobacterium* counts (log cfu/ml) in cow's, goat's and camel's milk containing different concentrations of suckary dates extract and inoculated with 1% culture of *B. infantis* when incubated at 37°C

Milk	Time (h)	suckary dates concentration %			
		0	10	15	20
Cow	0	7.13[1a]	7.18[a]	7.12[a]	7.24[a]
	4	7.73[a]	6.85[a]	6.68[a]	7.36[a]
	8	7.41[a]	7.47[a]	6.80[a]	6.68[a]
	12	6.97[a]	7.07[a]	7.31[a]	7.13[a]
	16	7.37[a]	7.29[a]	7.18[a]	7.48[a]
Goat	0	6.77[a]	6.76[a]	6.74[a]	6.79[a]
	4	6.73[a]	6.74[a]	6.78[a]	6.77[a]
	8	6.84[a]	6.83[a]	6.34[a]	6.82[a]
	12	6.83[a]	6.84[a]	6.83[a]	6.84[a]
	16	6.82[a]	6.83[a]	6.83[a]	6.83[a]
Camel	0	6.53[a]	6.55[a]	6.49[a]	6.53[a]
	4	6.60[a]	6.60[a]	6.81[a]	6.60[a]
	8	6.51[a]	6.87[a]	6.77[a]	6.51[a]
	12	6.69[a]	6.67[a]	6.72[a]	6.69[a]
	16	6.48[a]	6.73[a]	6.84[a]	6.48[a]

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

Table 4 : Changes in the pH of cow's, goat's and camel's milk containing different concentrations of suckary dates extract and inoculated with 1% culture of *Bifidobacterium infantis* when incubated at 37°C

Milk	Time (h)	Suckary dates concentration %			
		0	10	15	20
Cow	0	6.92[1a]	6.91[a]	6.91[a]	6.86[a]
	4	6.71[a]	6.76[a]	6.81[a]	6.84[a]
	8	6.69[a]	6.71[a]	6.77[a]	6.80[a]
	12	6.42[a]	6.42[a]	6.55[a]	6.77[a]
	16	5.24[a]	5.41[a]	5.92[a]	5.98[a]
Goat	0	6.79[a]	6.76[a]	6.72[a]	6.70[a]
	4	6.48[a]	6.41[a]	6.42[a]	6.43[a]
	8	6.44[a]	6.48[a]	6.49[a]	6.52[a]
	12	6.43[a]	6.36[a]	6.37[a]	6.27[a]
	16	6.16[a]	5.86[a]	5.81[a]	5.8[a]
Camel	0	6.73[a]	6.73[a]	6.75[a]	6.76[a]
	4	6.62[a]	6.6[a]	6.6[a]	6.61[a]
	8	6.57[a]	6.53[a]	6.58[a]	6.60[a]
	12	6.52[a]	6.47[a]	6.57[a]	6.56[a]
	16	6.17[a]	6.03[a]	6.05[a]	6.29[a]

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

indicates that the coagulation is not due to acidity but probably due to microbial enzymes. Goat's milk showed the highest occurrence of coagulation followed by camel's milk, whereas cow's milk showed low coagulation occurrence.

Table 5: Sensory evaluation[1] of cow's, goat's and camel's milk containing 10% of hilwa dates extract

Milk	Aroma	Taste	Color	Overall acceptability
Cow	3.6[2ab]	3.3[a]	3.3[bc]	3.4[a]
Goat	3.5[ab]	3.2[a]	3.1[c]	3.2[ab]
Camel	3.5[ab]	3.3[a]	3.6[bc]	3.2[ab]

[1]A 5 points-hedonic scale whereby 1 means dislike very much, 2 dislike, 3 neither dislike nor like, 4 like and 5 like very much.
[2]Values represented means (n=20). Means with different litters within a column are significantly different at (p<0.05).

The loss in viability of *Bifidobacteria* occurs in fermented milks due to several factors including acid and presence of oxygen (Shah, 2000).

Bifidobactria could grow well in milk inoculated with cultures prepared in a synthetic medium.

Retention of viability of *Bifidobacteria* was greatest with high amylose corn starch (hi-maize). The average pH of skim milk at the end of 4 weeks storage averaged between 4.34 (for *B. animals* with raftilose) to 4.07 (for *B. longum* with inulin). The highest levels of acetic acid and lactic acid were produced, respectively, (Bruno *et al.*, 2002).

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 .

Possible reasons for limited growth: The starter cultures selected for this study do not seems to be suitable for the growth in the different pure milks nor in the enriched milk preparations. Al-Saleh (2001) found similar results in camels milk, where he used *B. angulatum*, *B. infanti*, and four other species. A minor substantial growth was observed only in *B. Longum* whereas a substantial decrease in number was recorded in all other species. In contrast to that, Ustunol and Gandi (2001) observed doubling of the bacterial number of *B. Bifidum* cultured in skim milk enriched with sucrose, honey, fructose or glucose within at least 222 minutes in honey- enriched milk.

The non-encouraging results of the growth study of the two Bifidus species used, raise the question how the growth can be promoted? The use of other more suitable commercial pure or mixed culture may be most promising (Medina and Jordan, 1994). The use of additives such as cysteine (Biavati, 1992) amylose (Hughes and Hoover, 1995) or oligosaccharide (Roberfroid, 1998) may be practical. Another aspect to be tested is to ensure the prevalence of strict anaerobic

conditions. This could be accomplished by exposing the milk to high vacuum to expel soluble oxygen just before incubation.

In the light of the restricted growth of the *Bifidobacterium* species, the judgment regarding the suitability of the produced milk preparation, as a healthy drink, should be based on two aspects: the first one is the efficacy of the surviving bacterial number (> 10^6 cfu/ml) to reside in the intestinal estral and causing a positive health impact and the second is the sensory acceptance of the drinks. 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.

Regarding the sensory quality, the results of the sensory evaluation (Table 5) indicate a moderate acceptance for cow's milk preparations with hilwa dates extracts and less than moderate for camel's milk and the least acceptance for goat's milk preparation.

These results confirm reports which mention a low sensory acceptability of Bifidus milk (Al-Saleh, 2001). 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 cold storage of inoculated milks with Bifidobacteria revealed high rate of bacterial survival that encourage the production of Bifidus milk just by inoculation. On the other hand, the pH in both incubated and refrigerated treatments decreased gradually and sometimes with significant differences at ($p<0.05$ level), that indicating activity of the starter cultures used.

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