PRODUCTION AND PROPERTIES OF NON TRADITIONAL BIFIDO FERMENTED MILK ALONG THE STORAGE

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ABSTRACT

This study was carried out to investigate the influence of different milk types (cow, buffalo, goat and ewes' milk) on growth activity and viability of *Bifidobacterium bifidum, Bifidobacterium longum* and *Bifidobacterium catenulatum* along incubation and storage period. The basic hypothesis of this study was that fermented goat and ewe milk could possibly have a stronger stimulatory effect on the growth of *Bifidobacterium* strains than fermented cow and buffalo milk along incubation and storage period. The correlation between the stimulatory effect and some fermentation parameters (viable counts of *Bifidobacterium* strains and pH of fermented milk) was also analyzed. Viability of *Bifidobacterium* strains and pH values were also measured in milk types after 2, 4, 6, 8 and 12 h from the start of fermentation process.

The obtained results have shown a considerably higher stimulatory effect of fermented ewes' and goats' milk on the growth of tested *Bifidobacterium* strains as compared to that of fermented cow milk. At the same time, higher acidity and viability of *Bifidobacterium longum* were noted in fermented ewe and goat milk in all the phases of the incubation process. Also, *Bifidobacterium catenulatum* followed by *Bifidobacterium bifidum* have the maximum growth rate in both ewe and goats' milk. Chemical analysis of different Bifido fermented milk showed significant differences among milk type and refrigerated storage. Viscosity was milk type dependant and the storage had significant effect on viscosity. While, there were no significant differences among ewes' and buffalos' milk in sensory properties along refrigerated storage.

Sensory properties significantly enhanced using ewes' milk when fresh and along the storage period as compared with Bifido fermented milk produced from cows' milk. Consequently, a significant correlation between growth stimulation for *Bifidobacterium* strains and milk type was noted. Bifido fermented milk with high viable counts and better sensory properties could be produced from ewe and goats' milk.

Keywords: *Bifidobacterium*, viability, survival, milk type, Bifido fermented milk, storage.

INTRODUCTION

Egyptian consumers have progressive increase in their interest in health benefits for their dairy products, such as fermented milk. There has been an increase incidence of *Bifidobacterium* stimulation as a result of the probiotic cultures development to support and improve health. Probiotics and prebiotics have received much attention on their proclaimed potential health benefits to consumers. Today, *Bifidobacterium* species are the main probiotics used in production of fermented dairy foods with potential heath benefits. The Prolongation of life, that consumption of fermented dairy products by *Bifidobacteriium* resulted in improved health and longer life. The term "probiotic" refers to "microbial preparations that when ingested exert a positive influence on host health and physiology" (Ouwehand *et al.,* 1999; FAO/WHO, 2002; Tannock, 2002 and 2003). Among all probiotic microorganisms, *Bifidobacterium* are considered beneficial microorganisms because of their constructive health effects, as they maintain and create unfavourable conditions for pathogens growth (Biavati and Mattarelli, 2001 and Holzapfel and Schillinger, 2002).

Bifidobacteria can account 25% of the total numbers of predominant bacteria present on the colonic microflora. Bifidobacteria can also confer several other benefits on their host due to the shift in intestinal pH induced by the acidic metabolites during carbohydrate fermentation inhibits the growth of undesirable, potentially pathogenic bacteria (Gibson and Wang, 1994; Ballongue, 1998; Collins and Gibson, 1999; Schrezenmeir and de Vrese, 2001 and Piçard *et al.,* 2005). The proliferation of *Bifidobacterium* in milk is more difficult compared with that of conventional starters. Although bifidobacteria are considered more susceptible to oxygen than *L. acidophilus* due to their anaerobic nature, the oxygen susceptibility of bifidobacteria could however be strain dependent. Bifidobacteria survived well over a 35 day period in yoghurt, regardless of the oxygen content and redox potential of the yoghurt (Dave and Shah, 1997 and Miller *et al.*, 2002).

For probiotic bacteria to be effective however, it is essential that they reach the small intestine in sufficiently high numbers. It has been suggested that the daily intake should be at least 10^8 cfu for probiotic bacteria to deliver their therapeutic effects. (Lourens-Hattingh and Viljoen, 2001). Therefore the probiotic bacteria counts in probiotic yoghurts recommended being at least 10^6 cfu g^{-1} at the time of sale (Kurmann and Rasic, 1991 and Shah, 2001) and the probiotic strain must tolerate the hostile conditions of the gastrointestinal tract such as high acidity in the stomach and bile in the small intestine.

Although it has been suggested that probiotic microorganisms have the ability to exert potential heath benefits when damaged or non-viable (Salminen *et al.*, 1993; Shah *et al.*, 1995; Shah and Lankaputhra, 1997; and Salminen *et al.*, 1999), it is commonly established that most probiotic effects imply that the microorganisms are in viable state at the time of consumption. In fact, a requisite for a product to be considered probiotic is that the number of bacteria at the time of consumption is approximately 10^6 viable cells per gram of product (Sanders and Veld, 1999; and Boylston *et al.*, 2004) being the minimum recommended daily dose 10^8 to 10^9 cells (De Vuyst, 2000). Products containing probiotic microorganisms must therefore retain viability of these throughout the storage period. The decline in *Bifidobacterium* populations in a fermented milk product and was strain selection and pH dependant. Improving the viability of bifidobacteria and ensure that sensible numbers of bacteria are deliver to the host upon consumption after storage still needed.

It is easier to digest than cows' milk and may have certain therapeutic value. Goats' milk products, especially cheeses and yogurts, are very popular (Haenlein, 2004 and Vargas *et al.*, 2008). Production and application of goat and ewe's milk has been rising steadily, partially because of its good nutritional value and the nutritional benefits could be improved by adding probiotic species such as *Bifidobacterium*. Also, sheep and goat milk products can be an alternative to cow milk products owing to their specific taste, texture, their natural and healthy image. The addition of probiotic bacteria is made, not only because of certain claimed health potential and promoting benefits, but also because of sensory advantages, as well as the expanding variety of products that can be formulated with them (Vinderola, *et al.*, 2000 and Mutlu *et al.*, 2007). *Bifidobacterium* require long fermentation time, anaerobic conditions, low redox potential and exhibit weaker growth and acid production in fermented dairy products from cows' milk (Gomes and Malcata, 1999 and Kongo, *et al.*, 2006).

Research in incorporating and the growth of *Bifidobacterium* in ewes' and goats' milk and much information is still unavailable (especially, in Egypt) and most of the available research on the growth of *Bifidobacterium* in milk is related to cows' milk. Specific characteristics of ewes' and goats' milk in comparison with those of cows' milk have been reviewed (Park *et al.*, 2007 and Ljutovac *et al.*, 2008). Survival of *Bifidobacterium bifidum* was good in frozen yogurt, ice cream and probiotic cheese from goat milk (Holcomb *et al.*, 1991; Hekmat and McMahon, 1992 and Gomes and Malcata, 1998 and 1999).

The main objectives of this work were to determine viability and survival of *Bifidobacterium* strains in different fermented milk types using cow, buffalo, goat and sheep milk along incubation and refrigerated storage. Also, evaluate chemical, microbiological, rheological and organoleptic properties of non traditional bifido fermented milk along the storage.

MATERIALS AND METHODS

Milk and microbial strains:

Different types of fresh raw cow, buffalo, goat and ewes' milk were used throughout this study. Cow's milk was obtained from the herd of the instructional farm of High Institute for Agricultural Cooperation, Cairo-Alexandria desert road. Buffalo's milk was obtained from the herd of faculty of agriculture, Ain Shams University. Sheep (Barki) milk was obtained from Maryut Research Station, and Goat (Zaraybia and Shami) milk was obtained from Ras Sedr Research Station, Desert Research Center, Ministry of

Agriculture. Low heated spray dried skimmilk [VARIMEX Poland] was used throughout this work.

Three *Bifidobacterium* strains (*Bif. bifidum* ATCC15695 and *Bif. longum* ATCC 15707) were obtained from the Egyptian Microbial Culture Collection [EMCC] at Cairo Microbiological Resources Center (Cairo MIRCEN), Faculty of Agriculture, Ain Shams University. While, the third strain were Egyptian *Bifidobacterium* isolate identified as *Bifidobacterium catenulatum.*

Experimental procedure:

Preparation of skimmilk medium

Skimmilk medium was prepared according to Harrigan (1998). Skimmilk powder was reconstituted to 12% total solids with distilled water and sterilized at 121°C for 10 min, subsequently cooled to the incubation temperature, inoculated at level of 2% and incubated at 37°C for 16 h until coagulation (about pH 4.6) and used for activation and cultivation of *Bifidobacterium* strains.

Production of Bifido fermented milk

To investigate the growth characteristics of different three *Bifidobacterium* strains, different four raw milk types were autoclaved at 121°C for 10 min and subsequently cooled to 37°C and inoculated with 2% of single strain of activated *Bifidobacterium* culture. All treatments were incubated at 37°C for 12 h and *Bifidobacterium* counts and pH values were determined after 2, 4, 6, 8 and 12 h of incubation.

Bifido fermented milk products were produced using the four raw milk types (cow, buffalo goat and ewes' milk. Different row milk were heat treated (85°C for 15 min) and subsequently cooled to 37°C. Every type of milk were divided into three portions, and inoculated with 2% of single strain of activated *Bifidobacterium* culture. All treatments were incubated at 37°C till the coagulation (fermentation was stopped when fermented milk pH reached 4.6) then cooled and the resultant Bifido fermented milk were stored at 5±1°C for 14 days. Samples were taken when fresh and after 3, 7, 11, and 14 days of storage. The chemical, microbiological, rheological and sensory properties analyses were carried out along refrigerated storage.

Analytical methods:

1. Chemical properties:

The titratable acidity % (as lactic acid), and fat content were determined as described by Ling (1963). Values of pH were measured by using laboratory pH meter (Beckman electric pH meter) with combined glass electrode Model 3305 pH meter. The moisture content was determined using thermostatically controlled oven at 105°C for 3 h as described by AOAC, (2007). Ash content was determined according to the methods described by the AOAC, 2007 using muffle furnace [Thermolyne Type 1500] at 600 °C. The total nitrogen (TN) and soluble nitrogen (SN) contents of fermented milk samples were determined by the semi-micro Kjeldahl method according to the method described by AOAC, (2007). Acetaldehyde content was determined using Conway micro diffusion-Semi carbazide method according to Lee and Jago, (1969).

2. Microbiological and rheological quality:

Bifidobacteria count was enumerated using modified MRS agar supplemented with 0.05% L-cystein-HCl and 0.3% lithium chloride. The plates were anaerobically incubated (using anaerobic jars with gas pack BBL) at 37 °C for 48h (Dave and Shah, 1996; and Roy 2001).

The whey syneresis was determined as described by Dannenberg and Kessler, (1988). Apparent viscosity (at the shear rate of 57.6/sec) of fermented milk samples was measured and calculated according to Schaffner and Beuchat (1986) using a coaxial rotational viscometer (RHEOTEST 2-Germany) at shear rates ranging from 1 to 437.4 /sec. The measuring device (S2) was used with a samples volume of 30 ml per run. All samples were adjusted to the room temperature (23 ±1 °C) before loading it in the viscometer device. Apparent viscosity was estimated using an equation

$\eta_{app} = t / \gamma \times 100$ and $t \mp z \cdot \alpha\alpha$ where:

η_{app} = apparent viscosity (mpa.s), t = shear stress (dynes/cm^2), γ= shear rate (sec^{-1}), z = clyinder (constant S2) and α = read out value.

3. Sensory evaluation:

Organoleptic properties of Bifido fermented milk from different milk were assessed by a regular taste panel of the staff-members of the Food Science Department, Faculty of Agriculture, Ain Shams Univ. The samples were organoleptically assessed by six panellists, using a sensory rating scale of 1–10 for flavour and taste, and 1–5 for consistency and appearance, as described by Bodyfelt *et al.,* (1988). The evaluated organoleptic properties included: (a) six attributes for flavour and taste (nocriticism: 10; sour: 9; creamy: 9–7; sweet: 9–7; lack of flavour: 9–7; cooked: 9–6 and other: 5–1); (b) four characteristics of consistency (no criticism: 5; gel-like: 4–2; ropy: 3–1; too firm: 4–2 and too thin: 4–1) and (c) four terms describing appearance (no criticism: 5; atypical color: 4–2; lumpy: 4–2; shrunken: 4–1 and whey syneresis).

4. Statistical analysis:

The obtained results were analyzed using two-way analysis of variance (ANOVA) with a 95% confidence interval using ANOVA data and the general linear models procedure of SAS (Statistical Analysis System User's Guide SAS, 2000) (SAS Institute, Inc, U.S.A.). Duncan multiple tests was carried for multiple comparisons among means ($p<0.05$) of all the data.

RESULTS AND DISCUSSION

The data in Table (1) demonstrate the specific chemical composition of cows', buffalo's, ewes' and goats' milk used for the production of fermented milk. The chemical composition fell within the average: total solids 12.85 to 17.5%, fat 3.6 to 6.8%, protein 2.9 to 5.1%, lactose 4.81 to 5.01% and ash 0.7 to 0.89%. There were distinct significant differences between ewes', goats' and cows' milk. Buffalo's, ewes' and goats' milk contain a higher portion of total solids in comparison with cows' milk. Total protein and fat contents are the more quantitatively variable component of milk. Haenlein and Wendorff (2006) reported that, total protein content may vary from 2.6 g/l to 4.1 g/l for goat milk, and from 4.7 g/100 g to 7.2 g/100 g for ewe milk. The Ewes' and goats' milk contain a higher portion of smaller fat globules and medium chain fatty acids in comparison with buffalo's and cows' milk. The micelle structure of ewes' and goats' milk differs from that of cows' milk in average diameter, hydration and mineralization. Goats' milk has less casein than ewes' and cows' milk. Both goat and ewe milk micelles are highly mineralized and the size of caprine micelle is significantly higher than bovine milk (Pirisi *et al.*, 2007 and Ljutovac *et al.*, 2008).

Table (1): Chemical composition of raw cow, Buffalo's, goat and ewes' milk used to prcduce Bifido fermented milk.

Milk type	Total solids (%)	Fat (%)	Protein (%)	Lactose (%)	Ash (%)	pH	Titratable Acidity (%)
Cow	12.85± 0.22^{c}	3.6±0.2^{c}	2.9±0.10^{c}	4.81±0.12^{b}	0.70±0.04^{b}	6.68±0.04^{a}	0.16±0.01^{a}
Buffalo	17.5±0.15^{a}	6.8±0.2^{a}	5.1±0.14^{a}	4.92±0.03^{ab}	0.89±0.04^{a}	6.6±0.05	0.17±0.023^{a}
Goats	13.28±0.15^{b}	3.8± 0.2^{b}	3.6±0.12^{b}	5.01±0.05^{a}	0.76±0.03^{b}	6.58±0.04^{b}	0.17±0.01^{a}
ewes	16.89± 0.21^{a}	5.6±0.4^{a}	5.2±0.15^{a}	4.86±0.04^{ab}	0.83±0.05^{a}	6.61±0.05^{b}	0.16±0.02^{a}

$^{a,b\ and\ c}$ Values are given as means ± S.D (n=3).
Means of the same parameter with different small letter in the same column are significantly different ($p \leq 0.05$)

Changes in pH values and growth of *Bifidobacterium* strains along incubation period:

In order to investigate the influence of different milk types on the growth of three *Bifidobacterium* strains (*Bif. bifidum, Bif. longum* and *Bif. Catenulatum*), experiments using buffalo's, cows', ewes' and goats' milk were carried out. The reduction in pH values and viability of three *Bifidobacterium* strains during incubation is presented in Fig. (1). The data reveals that all bifidobacterial population could increase in all milk types. The increase in bifidobacterial population and the decrease in pH values along incubation period was milk type and strain dependant. The activity of the starter culture and rate of pH decline increased using ewes' followed by goats' milk and the maximum decrease in pH values was recorded in *Bifidobacterium catenulatum* followed by *Bifidobacterium longum.* Also, during incubation *Bifidobacterium* strains grew better in ewes and goat milk than in cow milk.

Generally, at the end of the incubation pH values ranged from 4.8 to 4.4 for fermented ewes' milk with *Bifidobacterium catenulatum* and fermented buffalo's milk with *Bifidobacterium bifidum,* respectively. The maximum *Bifidobacterium* counts were in fermented goats' and ewes' milk with *Bifidobacterium catenulatum* (9.6 and 9.85 log_{10} CFU/ml, respectively). On the contrary, the lowest *Bifidobacterium* counts were in buffalo's and cows' fermented milk (8.93 and 8.75 log_{10} CFU/ml, respectively). A considerable increase in population ($P \leq 0.05$) by two log cycles was observed in ewes' milk in 12 h of incubation followed by goats' and cows' milk. It can be seen that the counts were higher in ewes' milk and lower in cows' milk for all *Bifidobacterium* strains.

These data indicate that *Bifidobacterium* growing better in ewes' and goats' milk in comparison with buffalo's and cows' milk. Furthermore, *Bifidobacterium* growth was poorer in buffalo's milk as compared with all other milk types. Gomes and Malcata (1998) found higher *Bif. Lactis* counts in ewes' milk in comparison with goats' milk after 24 h of fermentation, when ewes' and goats' milk were evaluated for *Bifidobacterium lactis* and *Lactobacillus acidophilus* growth.

One of the major limitations of the incorporation of bifidobacteria into fermented milk is the pH of the product but, the pH optimum for bifidobacteria is between 6.5 and 7.0, with growth inhibited at pH values below 5.0 (Lourens-Hattingh and Viljoen, 2001). Because most strains of bifidobacteria are sensitive to pH values below 4.6, in practical applications, the pH value of the final product must be maintained above 4.6, otherwise the bifidobacterial population will decline rapidly (Vinderola *et al.,* 2002a and b). *Bif. breve* had ability to grow to probiotic levels (10^9 CFU/ml) when used as starter probiotic microorganism for the production of a potentially probiotic malt beverage. In goat and ewe's milk samples pH decreased faster than in cows' milk samples. In both milk types the growth of *Bifidobacterium catenulatum* was better in goat and ewe's milk samples than in buffalo cow's milk samples.

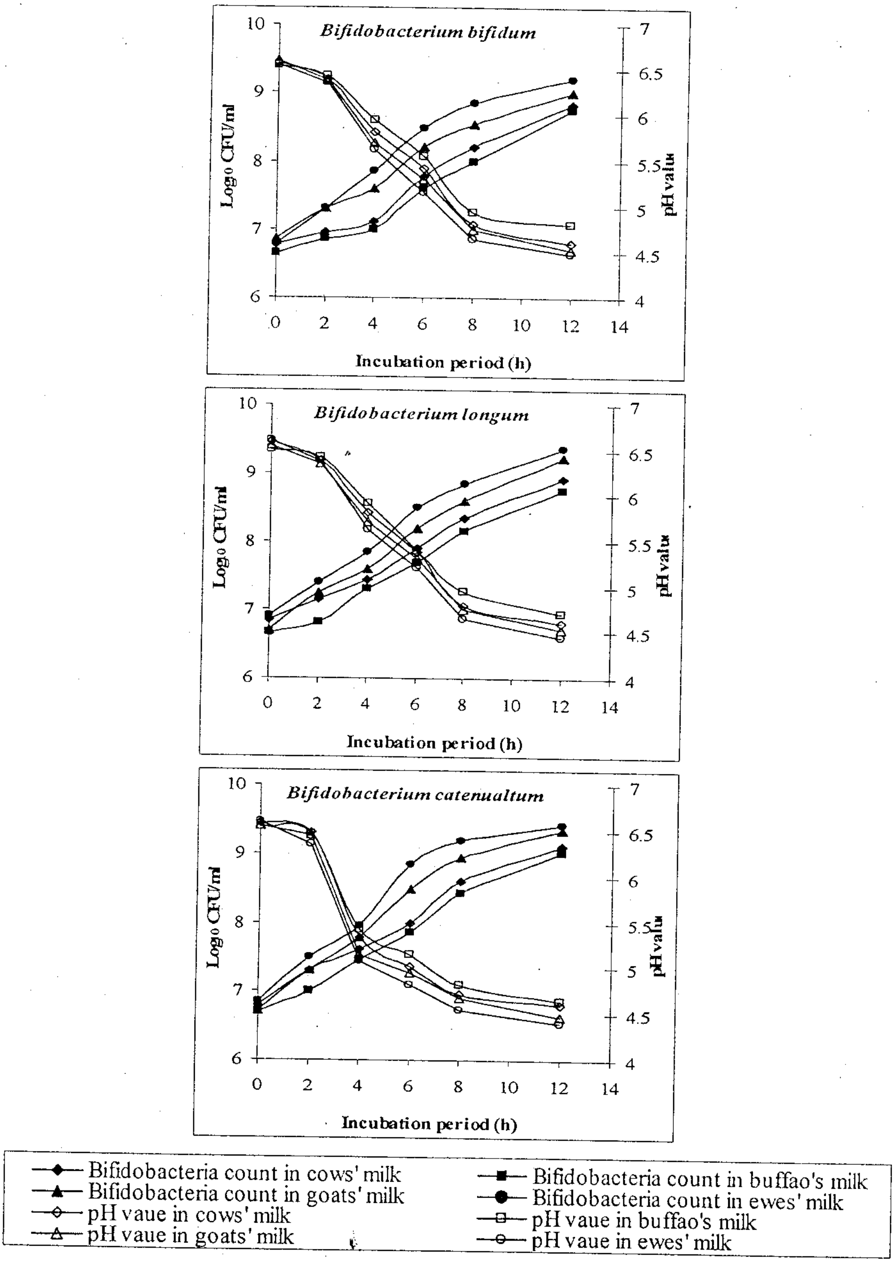

Fig. (1): Changes in pH values and viability (log_{10} CFU/ml) of *Bifidobacterium bifidum*, *Bifidobacterium longum* and *Bifidobacterium catenulatum* along incubation for 12 h in different milk types.

Table (2): Chemical properties of Bifido fermented milk when fresh and after 14 days of storage.

	Storage period (days)	Cow's milk			Buffalo's milk			Goat's milk			Ewe's milk		
		Bif. Bifidum	*Bif. Catenul*	*Bif. Longum*	*Bif. Bifidum*	*Bif. catenul*	*Bif. Longum*	*Bif. bifidum*	*Bif. catenul*	*Bif. longum*	*Bif. bifidum*	*Bif. catenul*	*Bif. longum*
Total solids (%)	Fresh	12.85^{a}	12.86^{a}	12.79^{a}	17.61^{a}	17.58^{a}	17.65^{a}	13.36^{a}	13.41^{a}	13.42^{a}	16.91^{a}	16.90^{a}	16.92^{a}
	14	12.99^{a}	13.01^{a}	12.95^{a}	17.69^{b}	17.66^{b}	17.74^{b}	13.45^{b}	13.47^{b}	13.51^{b}	16.98^{b}	16.97^{b}	16.99^{b}
Fat (%)	Fresh	3.6^{a}	3.6^{a}	3.6^{a}	6.6^{a}	6.5^{a}	6.5^{a}	3.8^{a}	3.7^{a}	3.8^{a}	5.6^{a}	5.7^{a}	5.8^{a}
	14	3.4^{a}	3.5^{a}	3.5^{a}	6.4^{a}	6.4^{a}	6.4^{a}	3.6^{a}	3.5^{a}	3.6^{a}	5.4^{a}	5.4^{a}	5.4^{a}
SN/TN (%)	Fresh	26.38^{a}	26.90^{a}	26.55^{a}	24.12^{a}	25.92^{a}	25.57^{a}	28.50^{a}	29.12^{a}	28.70^{a}	28.01^{a}	28.60^{a}	28.40^{a}
	14	29.21^{b}	29.71^{b}	29.52^{b}	28.51^{b}	28.75^{b}	28.54^{b}	31.28^{b}	31.11^{b}	30.51^{b}	30.50^{b}	31.11^{b}	30.95^{b}
Lactose (%)	Fresh	4.08^{a}	4.00^{a}	4.09^{a}	4.26^{a}	4.18^{a}	4.22^{a}	4.05^{a}	4.01^{a}	4.09^{a}	4.16^{a}	4.12^{a}	4.19^{a}
	14	4.01^{a}	3.96^{c}	3.98^{b}	3.79^{b}	3.71^{b}	3.75^{b}	3.61^{b}	3.52^{b}	3.64^{b}	3.81^{b}	3.62^{a}	3.84^{a}
Ash (%)	Fresh	0.74^{a}	0.75^{a}	0.70^{a}	0.94^{a}	0.93^{a}	0.93^{a}	0.77^{a}	0.78^{a}	0.78^{a}	0.88^{a}	0.86^{a}	0.85^{a}
	14	0.77^{a}	0.81^{a}	0.72^{a}	0.97^{a}	0.98^{a}	0.96^{a}	0.79^{a}	0.81^{a}	0.80^{a}	0.91^{a}	0.88^{a}	0.87^{a}

$^{a,b\ and\ c}$ Values are given as means (n=3).

Means of the same parameter with different small letter in the same column are significantly different ($p\leq0.05$)

The data in Fig. (2) illustrates the acetaldehyde content of Bifido fermented milk samples along storage for 14 days. Better acetaldehyde content of bifido fermented milk can be obtained using both ewes' and goats' milk as compared with using buffalo's and cow's milk. Acetaldehyde content slightly increased along the first week of refrigerated storage followed by gradual decrease till the end of the storage. The same trend was observed with the different types of *Bifidobacterium* strains. Bonczar *et al.,* (2002) and Bonczar *et al.,* (2004) stated that the type of starter culture used and the storage time influenced the acetaldehyde content and overall properties of the yoghurt and probiotic-fermented milk products.

Changes in pH values and survival of *Bifidobacterium* along refrigerated storage:

The data in Fig (3) presents the survival of *Bifidobacterium* strains and pH values along refrigerated storage of Bifido fermented milk samples. The data show statistically significant differences between non traditional milk types and cow milk in pH values and viability of *Bifidobacterium* along refrigerated storage .Also, refrigerated storage significantly affected the viability of *Bifidobacterium* strains and pH values.

In fresh samples, bifidobacteria counts varied from 8.1 to 9.2 $\log_{10}$ CFU/ml and pH values varied from 4.71 to 4.79. The data indicate that *Bifidobacterium* strains were growing and survived better in goats' and ewes' milk than in buffalo's and cow's milk along refrigerated storage for 14 days. Comparing the growth of *Bifidobacterium* strains it can bee observed that *Bifidobacterium catenulatum* had the highest viability along refrigerated storage. At the beginning of the storage, no expressive pH changes occurred between all strains. During refrigerated storage the pH of Bifido fermented milk samples from goats' milk decreased faster and they were lower (4.33, 4.3) than in Bifido fermented milk samples from cow's milk (4.37 to 4.35). It may be due to lower buffer capacity effect of goat's milk samples. These pH values are within the normal ranges for set-type yogurts. Misra and Kuila (1992) observed that the behaviour of *Bifidobacterium bifidum* varied with the type of milk (reconstituted skim milk, cow and buffalo milk).

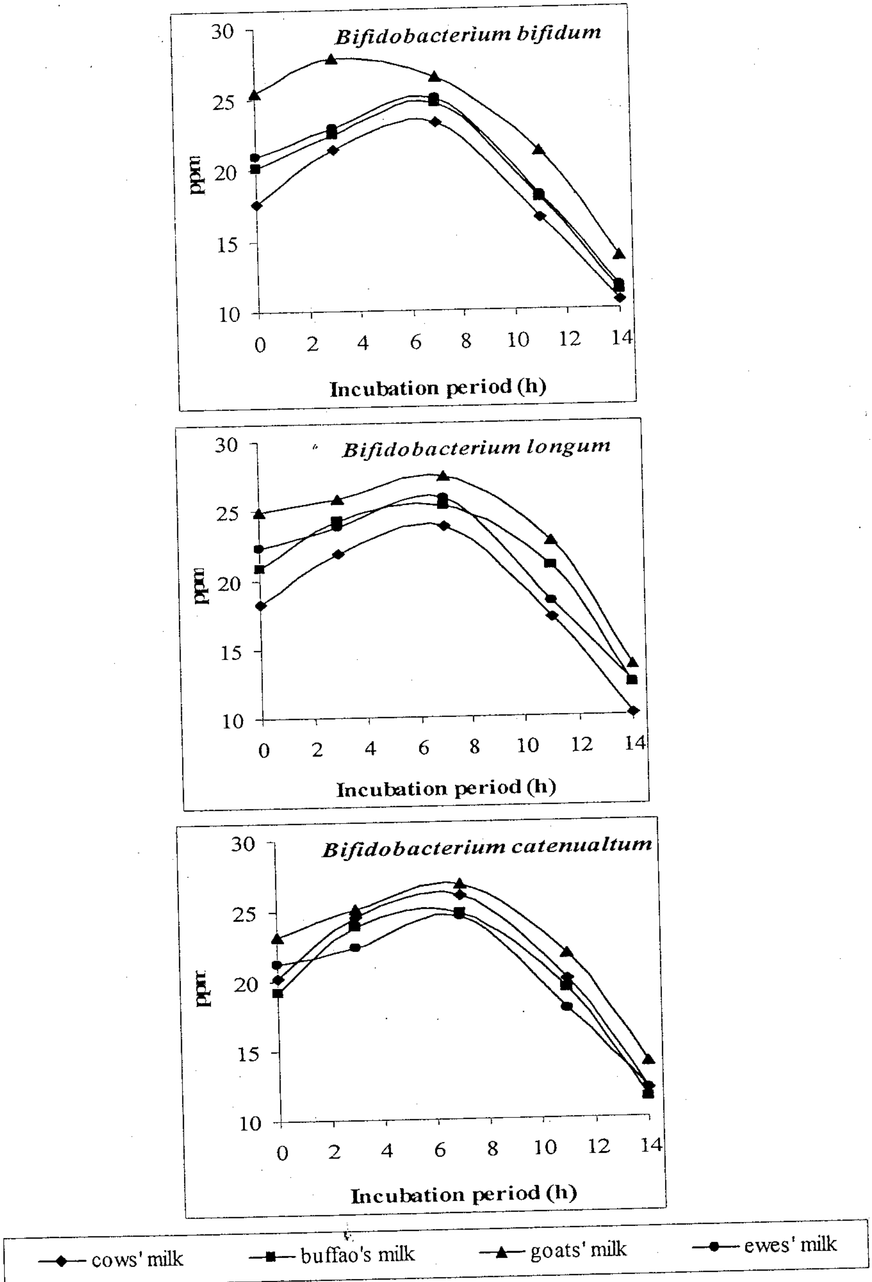

Fig. (2): Acetaldehyde content (ppm) of Bifido fermented milk along refrigerated storage (at 5±1°C/ 14 days).

The viable count of bifidobacteria at the beginning of the fermentation was milk type dependant and the viability of bifidobacteria was better in goat's and ewe's milk (Fig. 3). The survival of bifidobacteria was in all samples higher than the recommended level for probiotic fermented milk along refrigerated storage for 14 days. Generally, the viable count was higher in both Bifido fermented milk samples from ewes' and goats' having lower pH values as compared with Bifido fermented milk samples from buffalo's milk. The data was in agreement with those of Aumara and Farahat (2007) on synbiotic labneh along refrigerated storage.

Similarly, Rosental and Bernstein (1998) found that *Bifidobacterium bifidum* count was 7.63 log CFU/ml at pH 4.88 after 24 h of cow's milk fermentation at 37°C. Aso, Tabasco *et al.*, (2007) observed that, the viability of *Bifidobacterium lactis* gradually decreased in fermented milk to be more than 8.15±0.15 after 4 weeks of the storage at 4°C. Aumara and Hassan (2007) produced more healthy fermented milk with *Doum* palm fruit powder containing high viable bifidobacteria counts and Bifidobacteria count slightly increased along the 3rd day of the storage followed by a gradual decrease till the end of storage period but still higher than $1x\ 10^6$ cfu/ml.

Overall, the results have suggested a marked stimulatory effect of ewes' and goat's milk on viability of *Bifidobacterium* strains along storage period as compared with buffalo and cow milk. The numbers of *Bifidobacterium* strains are in considerable correlation with the use of ewe and goat milk in production of Bifido fermented milk. Therefore, it could be recommended that, Bifido fermented milk with acceptable sensory properties and high viable counts of *Bifidobacterium* sp. could be achieved using ewe and goats' milk.

Rheological properties Bifido fermented milk along the storage (at 5±1°C/ 14 days):

Since the apparent viscosity of Bifido fermented milk with buffalo's and ewe's milk was significantly high along the storage followed by Bifido fermented milk with goats' milk. Milk from ewes' and goats' milk may be good carriers for production of Bifido fermented milk with high apparent viscosity as compared with other different probiotic fermented milk samples.

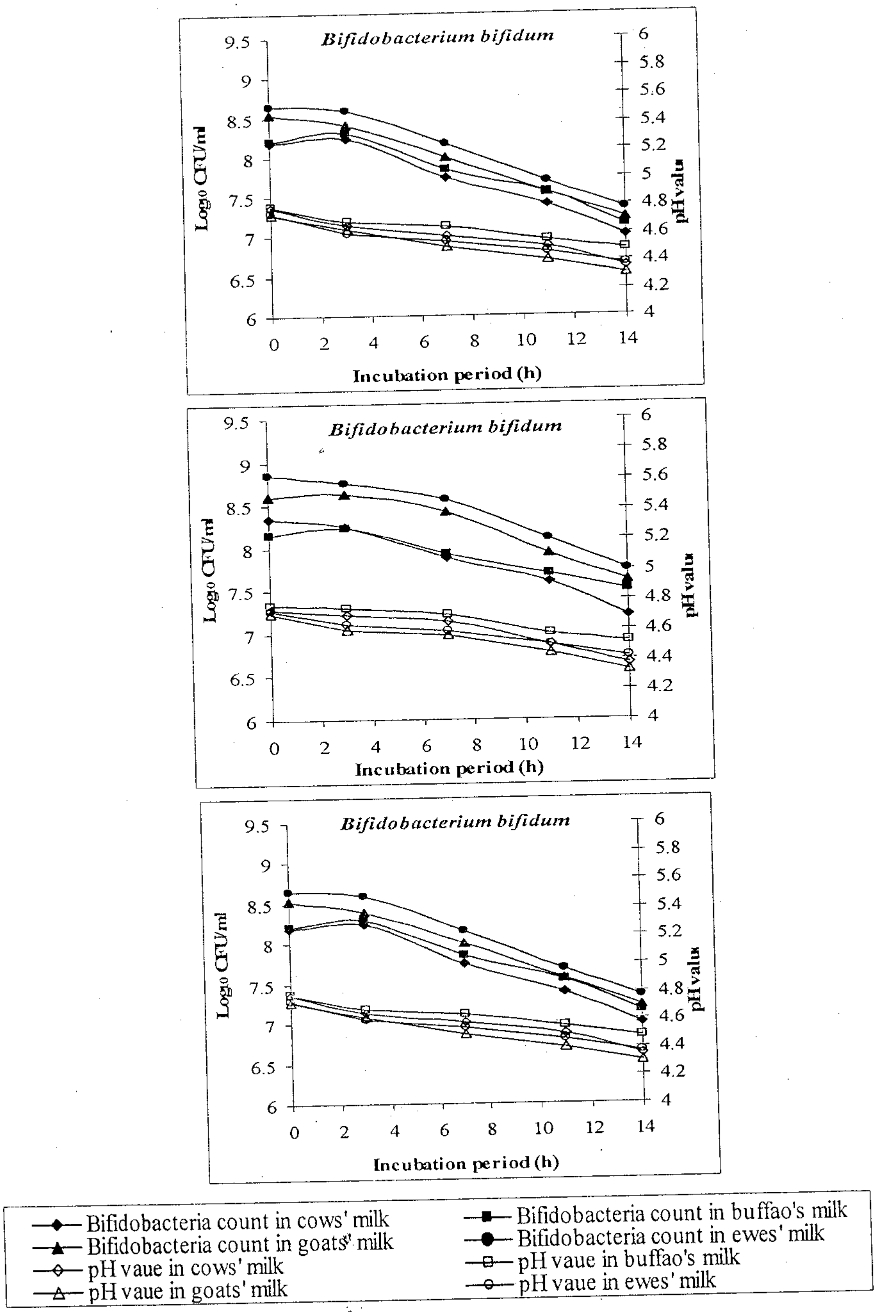

Fig. (3): Survival of *Bifidobacterium* strains (log_{10} CFU/ml) and pH values of Bifido fermented milk along refrigerated storage (at 5±1°C/ 14 days).

It appears that, the high apparent viscosity for Bifido fermented milk with buffalo's may be due to the high total solids content which have the main effect on viscosity.

Also, the apparent viscosity was strain and milk type dependant along refrigerated storage. Generally, apparent viscosity gradually increased as storage period progressed. Bifido fermented milk with buffalo's milk had the highest apparent viscosity. It may be due to the high total solid content and the high size for its protein particles which increase the holding capacity. On the contrary, fresh and stored Bifido fermented milk from cows' milk had the minimum apparent viscosity. Rhom and Kovac, (1994) established the differences in the rheological properties of yoghurts prepared by different starter cultures, which could be attributed to structural phenomena and to the magnitude of the relaxation modules of the gel strands as well as the dynamics of network formation in acid milk gel.

Fig. (5) illustrate the changes in whey syneresis of Bifido fermented milk samples along refrigerated storage. The data reveal that, whey syneresis was influenced by milk type and storage period. All samples had significantly low whey syneresis as compared to control samples (cows' milk). Generally, the use of ewes' and buffalo's milk significantly decreased the total whey syneresis of fresh and stored Bifido fermented milk samples. On the other hand, whey separation was greatest in samples from cows' milk and least in samples from buffalo's milk. The high levels of pH values in Bifido fermented milk samples from ewes and buffalo's milk had great effect on the whey syneresis.

Tamime and Robinson (1999) stated that the syneresis in yoghurt samples decreased during storage. This could be due to metabolic activity of yoghurt starter along the storage and to decrease in net pressure in the protein matrix, which decrease the whey syneresis.

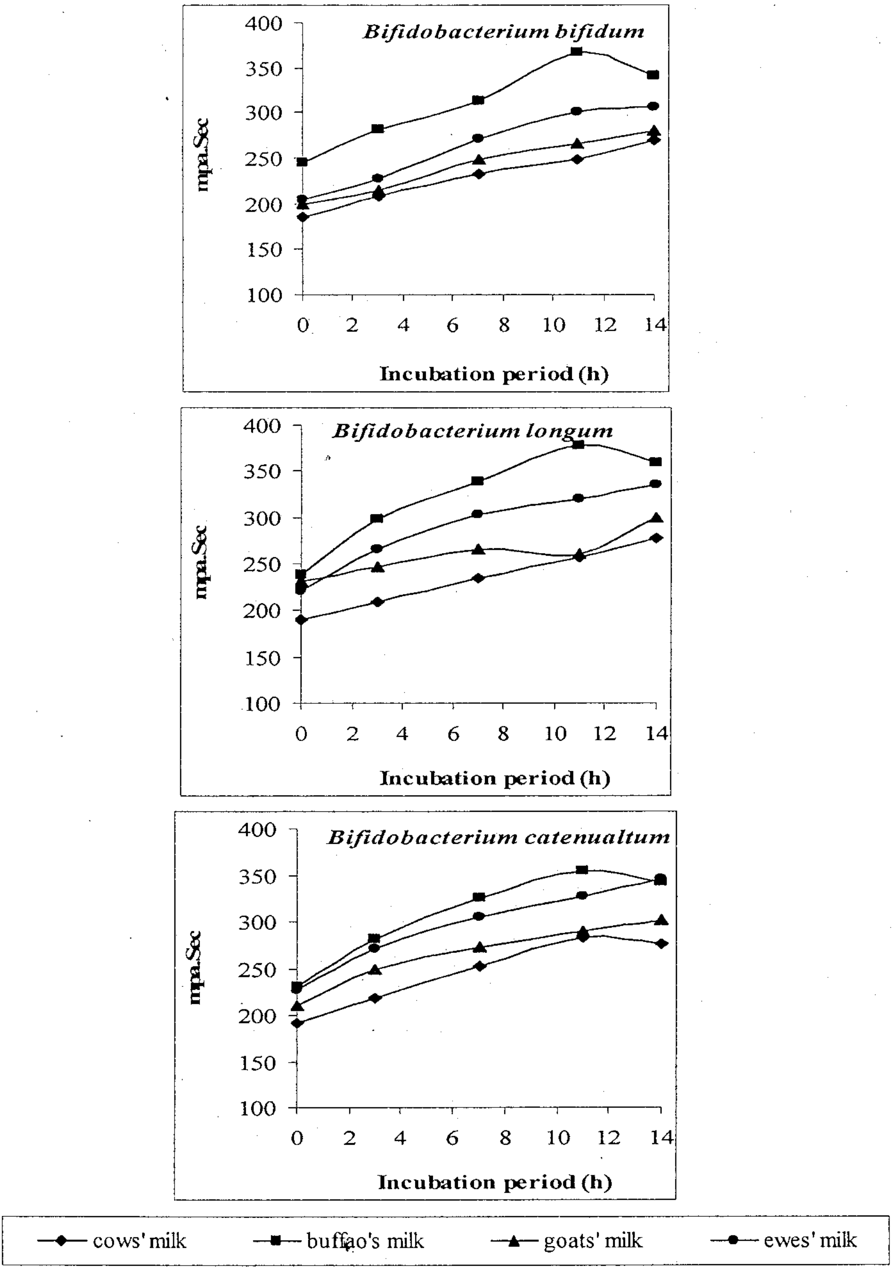

Fig. (4): Apparent viscosity (mpa sec^{-1}) of Bifido fermented milk along the storage (at 5±1°C/ 14 days).

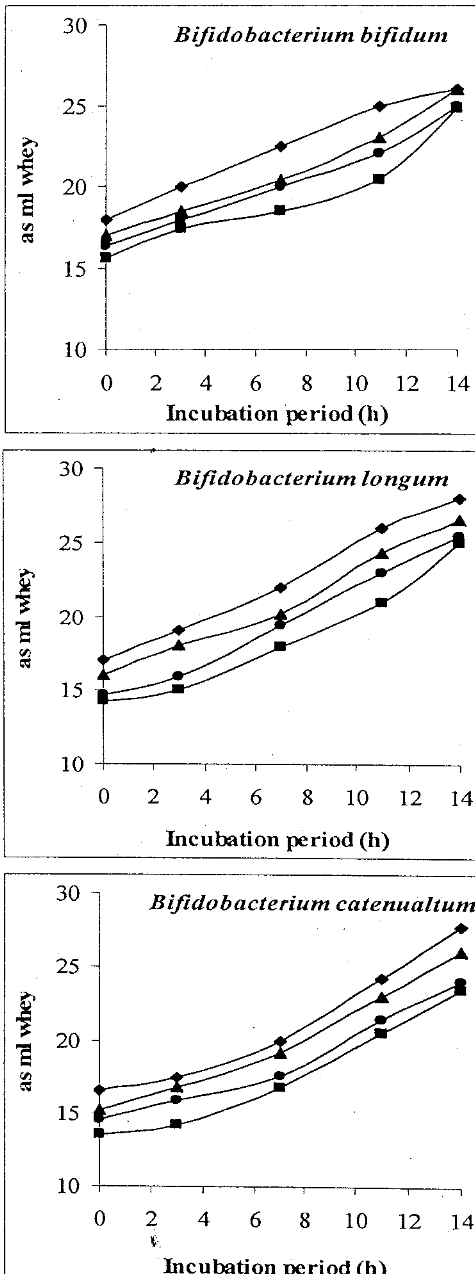

Fig. (5): Whey syneresis (as ml whey 25 g^{-1}) of Bifido fermented milk along refrigerated storage (at 5±1°C/ 14 days).

Sensory evaluation:

Bifidobacterium catenulatum showed the highest viability in Bifido fermented milk along refrigerated storage so it had been used for manufacture of Bifido fermented milk and sensory evaluated. Sensory properties of Bifido fermented milk samples along refrigerated storage are shown in Table 3. The expert panel indicated a preference for Bifido fermented milk from buffalo's and ewes' milk followed by goats' milk. Also, the milk type significantly influenced the sensory scores of Bifido fermented milk. While, there were no significant differences among ewes' and buffalos' milk along refrigerated storage. Bifido fermented buffalo's and ewes' milk were preferred over the other types of Bifido fermented milk samples when fresh and along the storage period. This could be related to the high acetaldehyde content and acidity of the samples. Mutlu *et al.*, (2007) stated that, yogurt appeared to be more acidic than the bio-yogurt, so it received lower organoleptic scores than the bio-yogurts after 14 days of the storage.

However, Bifido fermented cows' milk received significantly lower flavour scores than did the others during refrigerated storage. This maybe due to the lowest total solid content in cows' milk and the high fat content in both buffalo's and ewes' milk. While, low flavour scores for Bifido fermented goats' milk was due to the goat flavour which appears in fermented milk at the beginning of the storage and disappeared along refrigerated storage. Aumara and Hassan (2007) stated that organoleptic evaluation scores of probiotic more healthy fermented milk made with *Bif. longum* and *L. rhamnosus* culture was slightly higher than that made with *Bif. longum* and lactococci culture.

Vargas *et al.*, (2008) reported that the higher the goats' milk content, the greater the physicochemical and sensory differences with regard to the 100% cows' milk yoghurt. Also, samples with half and half cows'/goats' milk were preferred by the sensory panel and incorporating goats' milk had a significant impact on the whiteness, flavour, syneresis and lumpiness of yoghurts.

Therefore, it could be recommended that the use of ewes' and goats' milk had a significant effect to enhancement the fermented milk produced with *Bifidobacterium* probiotic strains. Also, the use of *Bifidobacterium catenulatum* for production of non traditional

Bifido fermented milk from ewes' and goats' with significant high bifidobacteria viable counts when fresh and along refrigerated storage for 14 days.

Table (3): Sensory properties of Bifido fermented milk manufactured with *Bifidobacterium catenulatum* along refrigerated storage.

	Storage period (days)	*Bifidobacterium catenulatum*			
		Cows' milk	Buffalo's milk	Goats' milk	Ewes' milk
Flavour	Fresh	8.0±0.3 ^{b}	8.8±0.1 ^{a}	7.8±0.2 ^{b}	8.7±0.1^{a}
	3	8.7±0.1 ^{b}	9.2±0.2 ^{a}	8.5±0.1 ^{b}	9.0±0.2^{a}
	7	8.5±0.2 ^{b}	8.8±0.1 ^{a}	8.5±0.1 ^{b}	8.7±0.2^{a}
	11	7.6±0.1 ^{c}	8.2±0.1 ^{a}	7.8±0.1 ^{b}	8.1±0.2^{a}
	14	7.1±0.3 ^{b}	7.5±0.2 ^{a}	7.6±0.2 ^{a}	7.5±0.1^{a}
Consisency	Fresh	3.5±0.3 ^{c}	4.7±0.1 ^{a}	4.3±0.2	4.6±0.2^{a}
	3	3.7±0.2 ^{c}	4.9±0.2 ^{a}	4.6±0.1	4.8±0.2^{a}
	7	3.8±0.4 ^{c}	4.8±0.2 ^{a}	4.7±0.2	4.9±0.2^{a}
	11	3.6±0.4 ^{c}	4.5±0.3 ^{a}	4.5±0.2	4.8±0.3^{a}
	14	3.4±0.3 ^{c}	4.1±0.1 ^{b}	4.1±0.3 ^{b}	4.4±0.3^{a}
Appearance	Fresh	4.4±0.2 ^{b}	4.9±0.3 ^{a}	4.5±0.1 ^{b}	4.9±0.2^{a}
	3	4.6±0.3 ^{b}	4.9±0.3 ^{a}	4.6±0.1 ^{b}	4.8±0.3^{a}
	7	4.3±0.3 ^{c}	4.7±0.2 ^{a}	4.6±0.2 ^{a}	4.5±0.1^{a}
	11	4.1±0.1 ^{c}	4.5±0.2 ^{a}	4.2±0.2 ^{b}	4.5±0.2^{a}
	14	3.8±0.1 ^{b}	4.3±0.1 ^{a}	4.2±0.2 ^{a}	4.3±0.1^{a}
Total scores	Fresh	15.9±0.4 ^{c}	18.4±0.3 ^{a}	16.6±0.2 ^{b}	18.2±0.3^{a}
	3	17.0±0.3 ^{b}	19.0±0.2 ^{a}	17.7±0.2 ^{b}	18.6±0.3^{a}
	7	16.9±0.2 ^{b}	18.3±0.3 ^{a}	16.8±0.3 ^{b}	18.1±0.2^{a}
	11	15.2±0.4 ^{c}	17.2±0.3 ^{a}	16.5±0.2 ^{b}	17.3±0.2^{a}
	14	14.2±0.2 ^{c}	15.8±0.1 ^{b}	15.9±0.2 ^{b}	16.2±0.2^{b}

Mean value of 6 panelist ± standard deviation
Means of the same parameter with different small letter in the same column are significantly different ($p \leq 0.05$)

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إنتاج وخواص البان متخمرة غير تقليدية باستخدام *Bifidobacterium* خلال التخزين المبرد

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تم إنتاج البان متخمرة بواسطة ثلاثة سلالات مختلفة هى:

Bifidobacterium bifidum و *Bifidobacterium longum* و *Bifidobacterium catenulatum*

وهذا باستخدام كلا من لبن ماعز و لبن أغنام وكذلك لبن جاموسى وإستخدم اللبن البقرى كمقارنة وقد تم متابعة التغيرات الكيماوية والميكروبيولوجية لها خلال فترة التحضين (لمدة ١٢ ساعة) وكذلك خلال التخزين المبرد لها كما تم متابعة التغيرات فى كلا من اللزوجة الظاهرية والقدرة على طرد الشرش لهذه الألبان المتخمرة. كما تم التحكيم الحسى على المنتج المصنع بإستخدام سلالة *Bifidobacterium catenulatum* حيث أنها كانت أفضل السلالات حيوية ونشاطاً خلال فترى التحضين والتخزين المبرد حيث تميزت المنتجات بالتالى:

كانت أفضل المعاملات هى تلك الألبان المتخمرة المصنعة من ألبان الماعز والأغنام فى درجة نشاط سلالات *Bifidobacterium* مقارنة بتلك الألبان المصنعة سواء من اللبن الجاموسى أو اللبن البقرى، حيث كانت أعداد سلالات *Bifidobacterium* مرتفعة فى كلا من ألبان الماعز والأغنام مقارنة باللبن البقرى والجاموسى. وتطورت حموضة الألبان خلال فترة التحضين وتأثر هذا التطور بنوع اللبن حيث كان لبن الماعز يليه لبن الأغنام أفضل أنواع الألبان كبيئات لنمو سلالات *Bifidobacterium* يليها لبن الأبقار وأخيراً اللبن الجاموسى.

بدراسة أعداد *Bifidobacterium* المتبقية خلال فترة التخزين المبرد وجد أن أعدادها كانت مرتفعة خلال فترة التخزين فى كلا من اللبن المتخمر المصنع من لبن الأغنام يليه المنتج المصنع من كلا من ألبان الأغنام أوالجاموس. وكانت الخواص الريولوجيه (اللزوجة وقدرة الخثرة على طرد الشرش) وكذلك الحسية أفضل ما يمكن فى كلا من اللبن المتخمر المصنع من ألبان الأغنام أوالجاموس خلال فترة التخزين المبرد.

لذلك يمكن التوصية بتصنيع لبن متخمر ذو خواص وتأثيرات صحية يحتوى على أعداد مرتفعة من *Bifidobacterium* وذو خواص جسية وتأثيرات صحية خلال التخزين المبرد على ٥°م لمدة ١٤ يوم باستخدام ألبان الأغنام أو الماعز المتخمرة.