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Full Length Research paper

Microbiological and biochemical changes and sensory evaluation of camel milk fermented by selected bacterial starter cultures

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The microbiological and biochemical changes that occur during fermentation of camel milk inoculated with each of five selected starter cultures at 43ºC for 6 h, were studied as well as the sensory evaluation of the products. The total viable counts of the starter cultures throughout fermentation period (6 h) showed that the combination of *Lactobacillus bulgaricus* CH2 plus *Streptococcus thermophilus 37* (1:1) had more counts and produce more acid (lower pH) compared to the single starter cultures. Also when comparing the different treatments, the amount of FAG released after 6 h was highest in the mixed starter cultures than in the corresponding single starter cultures. The final fermented milk products were free from pathogenic bacteria such as *Salmonella spp*, *Staphylococcus aureus*, *Listeria monocytogenes, Escherichia coli O157:H7* and *Bacillus cereus,* while the total coliforms, yeasts and molds counts were less than 10 cfu per ml. The results of the sensory evaluation study indicated that the camel milk fermented by mixed yogurt culture was the most accepted while the one fermented by *Lactococcus lactis* was the least. However, the consistency of all fermented camel milk products was watery and showed a fragile, poor structure (poor scores). In general mixed yogurt culture showed superior growth, acid production and proteolytic activity than single starter cultures and acceptable fermented camel milk.

Key words: Cultures, pathogenic bacteria, yeast, molds, camel milk.

INTRODUCTION

Camels (*Camelus dromedaries*) belong to the family *Camelidae* and the sub-order *tylopoda*. Sudan has the second largest number of camels in Africa. The population of camels in Sudan was estimated to be 2.8 million (FAO, 1990) distributed around the country. They belong to the one-humped dromedary kind, which originally reached the country from Arabia. The average daily milk yield of camels in Sudan was found to be 5 - 10 Kg (El-Amin, 1979). The Ministry of Animal Resources (1996) gave an estimate of annual milk production in Sudan of about 7.58 million tons of which 0.033 million tons is camel's milk. Camel milk is extremely popular and widely consumed by nomadic tribes in Sudan both as fresh raw milk and as soured milk especially in the East and West regions. Recent scientific and technological advances show that the nutritional and therapeutic importance of fermented dairy products had been attributed to the use of lactic acid cultures in their manufacturing process and to numerous metabolites and enzymes produced that possess some therapeutic benefits (Shahani and Chandan, 1979).

Abu Tarboush (1996) reported that the proteolytic activities of yogurt starters (at 42ºC for 4 h) were higher in

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camel milk than in cow milk. Fermented camel milk products have various names in various parts of the world, in Sudan *gariss* is a special kind of fermented camel milk popular among the nomads of Sudan, it is prepared by fermenting the camel milk in large skin bags or si'ins, which contain a large quantity of a previously soured product (Dirar, 1993). *Suusac* is fermented camel milk widely consumed by the pastoralist communities living in Kenya and Somalia. It is prepared by fermenting fresh camel milk in a pre-smoked gourd naturally at ambient temperature (26 - 29°C) for 1 - 2 days (Lore et el., 2005). Other researchers such as, Mehaia (1993) reported that cheese made from 100% camel milk has lower yield and lower component recovery than cheese made from cow milk.

Mohamed et al. (1990), observed that camel milk failed to form gel like structure after 18 h incubation with lactic acid culture, this was attributed to the presence of antibacterial factors such as lysozymes, lactoferrin immunoglobulin in camel milk (El Agmy et al., 1992). Farah et al. (1990) studied the preparation and consumer acceptability tests of fermented camel milk *(Suusa*). They found that the consistency of fermented milk (under lab conditions) was thin and a precipitate in the form of flocks was formed rather than a coagulum after fermentation. These reports clearly show the difficulty of producing fermented camel milk products with high consistency due to the problem associated with milk coagulation. Therefore, the objective of this study was to develop fermented camel milk by using selected pure starter cultures, in addition to study the microbiological and biochemical changes in the camel milk during the fermentation period.

MATERIALS AND METHODS

Sources and maintenance of cultures

Lyophilized pure culture strains of *Streptococcus thermophilus 37* , *Lactobacillus delbrueckii sp. bulgricus CH2, Lactococcus lactis, Lactobacillus acidophilus* and mixed yogurt culture (*S. thermophilus* and *L. bulgaricu* 1:1) used in this study were obtained from Chr. Hansen's Laboratorium. (Hørsholm, Denmark A/S). The working cultures were prepared by 100 mg of lyophilized cultures to 100 ml of previously reconstituted and sterilized (121ºC/15 min) skim milk with total solids of 11% and incubated overnight at 25ºC. One percent inocula from overnight coagulated cultures were propagated for three times before being used in growth studies. Cultures were grown and maintained in sterile reconstituted non-fat dry milk (NDM) containing 11% solids (wt/wt) with weekly transfers. Purity of cultures was routinely checked by performing Gram stains.

Preparation of fermented milk

Fresh whole camel milk from *C. dromedarius* was obtained from a private herd. Milk was immediately cooled and kept at 5 ± 1°C during transportation to the laboratory. The whole camel milk was pasteurized in 500 ml quantities at 80°C for 15 min in a water bath and cooled immediately to 5 ± 1°C in an iced bath. The milk samples (500 ml) were equilibrated for one hour at the fermentation temperature (43ºC) in a water path before inoculation with the starter cultures. The cultures were sub-cultured using 1% inocula (10^6 - 10^7 cfu/ml) in sterile 11% reconstituted non-fat dry milk (NDM) and incubated at 37ºC for 18 – 24 h at least three times before experimentation involving camel milk as the medium of growth. Each milk was inoculated with 5% (10^6 - 10^7 cfu/ml) of *S. thermophilus 37, L. delbrueckii ssp. bulgaricus CH2, L. lactis, L. acidophilus* and mixed yogurt culture (*S. thermophilus and L. bulgaricus* 1:1). The contents were thoroughly mixed after inoculation and incubated at 43°C in a shaker water-bath for 6h. Fifty ml of samples were taken in sterile bags aseptically for microbiological and biochemical tests every one and half hour. The final products of fermented camel milk after 6 h of incubation were analyzed for microbiological quality and sensory evaluation. The experiment was repeated three times.

Microbiological analysis

Fermented camel milk samples (11 ml) were homogenized for one minute in 99 ml ($^1/_{10}$) of a sterile solution of 0.1% (w/v) peptone water (Oxoid CM9) using a Stomacher Lab blender (Model400,Seward Laboratory, London). From these samples serial decimal dilutions were prepared in sterile 0.1% peptone water. The microorganism's counts were carried out by the pour-plate method with duplicate plating on different selective agar media (Parrow, 1978). The coliforms were estimated in duplicate pour plates of Violet Red Bile Agar (VRBA, Oxoid CM107) medium and the plates were overlaid after solidification with 3 to 4 ml of additional Violet Red Bile Agar. All plates were incubated in an inverted position at 30°C ± 2 for 18 – 24 h (Mehlman, 1984). The yeasts and molds were counted on acidified Potato Dextrose Agar, (Oxoid CM139) which was acidified by the addition of the proper amount of sterile 10% tartaric acid (Fluka-AG-Buchs.SG), then the plates were incubated at 25°C ± 1 for 3 - 7 days (Koburger and Marth, 1984).

The lactic acid bacteria were enumerated in pour plates of de Man, Rogosa and Sharp (MRS) medium (Oxoid CM359). The plates were incubated at 37℃ for 48 h under microaerobic conditions using Gas Pak (H_2+CO_2) (BBL, Microbiology Systems, Div. Becton Dickinson and Co., Cockeysville. Med.) anaerobic systems (Gilliland et al., 1984). The detection of *Salmonella spp., Staphylococcus aureus, Bacillus cereus, Listeria monocytogenes* and *Escherichia coli O157: H7* were applied according to the methods described in the FDA (1998).

Measurement of pH and titratable acidity

The pH was determined by inserting a pH probe (Orion Research Inc., Cambridge, MA, USA) directly into a homogenized sample of the fermented camel milk. Between samples, the electrode was rinsed with distilled water and wiped with tissue. The titratable acidity (expressed as lactic acid %) was determined by titrating 10 ml of homogenized fermented camel milk with 0.1 N NaOH to the phenolphthalein end point.

Measurement of proteolytic activities

The proteolytic activities of the cultures were determined spectrophotometrically, by additions of 10 ml of 0.75N TCA and 1 ml of water to 5 ml of sample to give a final concentration of 0.47N (7.7%) TCA. The samples were filtered using Whatman number 2 filter paper (Whatman Corp.Clifton, NJ) after 10 min of incubation at room temperature (25ºC). The O-phthaldialdehyde (OPA) method described by Church et al. (1983) was used to determine the concentration of free amino group (FAG) in the filtrate.

Sensory evaluation

Consumer acceptability of the different fermented camel milk pro-

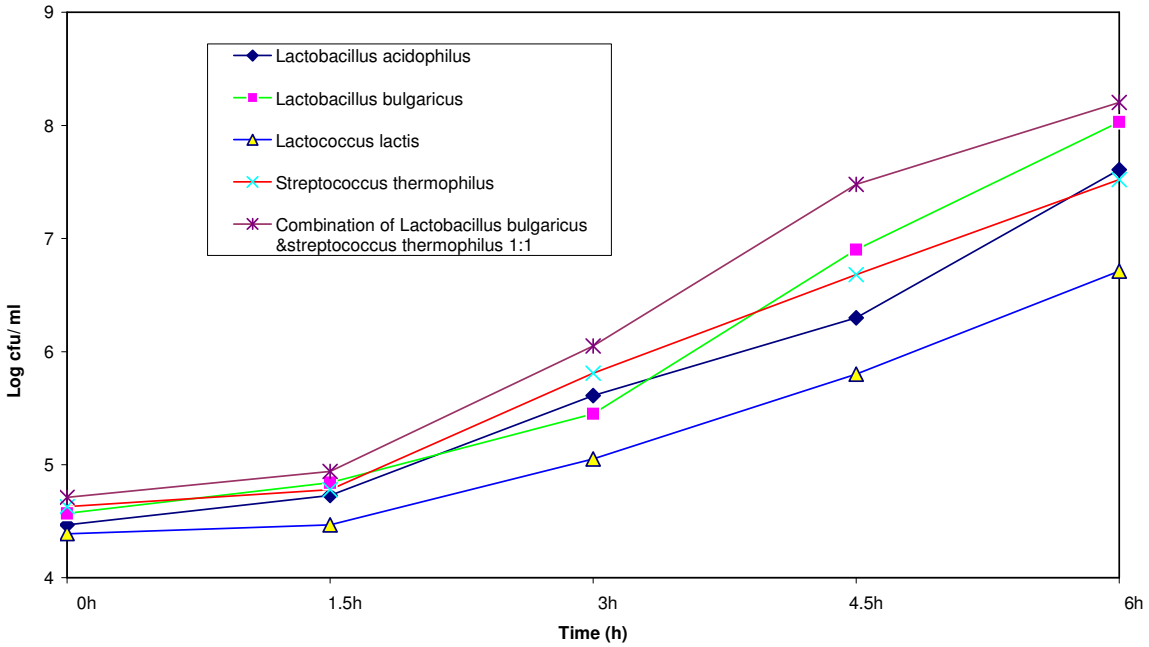

Figure 1. Changes in the viable counts of the starter culture strains during fermentation of camel milk for 6 h at 43°C.

ducts were evaluated by 10 consumer panelists (all of them were familiar with fermented camel milk-Gariss), using a 9-point hedonic rating scale (9 = excellent; 1 = extremely poor). The samples were evaluated for color, smell, taste, consistency and overall acceptability, also the panelists were asked to list any defects.

Statistical analysis

Each sample was analyzed in triplicate and the figures were then averaged. The statistical analysis was performed with SAS program (SAS, 1990) using analysis of variance (ANOVA) and means were separated by Duncan's multiple range tests with a probability $P \leq 0.05$ (Steel and Torrie, 1980).

RESULTS AND DISCUSSION

Microbiological analysis

The viable counts of starter cultures during fermentation

Changes in the viable counts of the starter cultures of lactic acid bacteria throughout fermentation are presented in Figure 1. The initial viable cell counts of starter cultures ranged from 4.39 (*L. lactis*) to 4.7 $\log_{10}$ cfu^{-ml} (combination of *L. bulgaricus* and *St. thermophilus* 1:1). These numbers indicated that the initial counts for the inoculated camel milk before fermentation were similar in the five cultures and remained stable with minor increase after 1.5 h of incubation. The average counts after 3 h incubation were 5.41, 5.65, 5.05, 5.51, 6.05 and those after 4.5 h were 6.3, 6.9, 5.8, 6.68, 7.48 $\log_{10}$ cfu^{-ml} for *L. acidophilus, L. bulgaricus, L. lactis*, *St. thermophilus* and combination of *L. bulgaricus* and *St. thermophilus* (1:1), respectively. At the end of the fermentation process (6 h) the counts increased to 7.61, 8.03, 6.71, 7.52 and 8.2 $\log_{10}$ cfu^{-ml} for the five culture strains respectively. Throughout fermentation period the bacterial populations for the all cultures increased and *L. bulgaricus* showed the fastest growth of all single cultures. Moreover, at any given time period counts of *L. bulgaricus* were always more numerous than the other single strains while those counts of *L. lactis* were least numerous. The total viable counts of the combination of *L. bulgaricus* plus *St. thermophilus* revealed more counts at the end of fermentation period (6 h) compared with single cultures. In contrast to this finding Abu-Tarboush (1996) found that the streptococci were always more numerous than the lactobacilli during fermentation of camel milk at 42ºC for 4 h.

On the other hand Abdel Moneim et al. *(*2006) have shown the predominance of lactic acid bacteria in garris product (Sudanese traditional fermented camel milk) and the major genus was *Lactobacillus* (74%). Also Lore et al. (2005) investigated suusac (Kenyan traditional fermented camel milk) and found the total lactic acid bacteria counts were 6.8 $\log_{10}$ cfu/ml and the main genus was *Lactobacillus spp.*

Microbiological quality of fermented camel milk products

The prevalence of *Salmonella spp.*, *S.s aureus*, *L. monocytogenes, B. cereus, E. coli O157:H7,* total yeasts and

Table 1. Microbiological analysis of camel milk fermented at 43ºC for 6 h.

Tests	Camel milk fermented at 43ºC for 6 h by:				
	Lactobacillus acidophilus	***Lactobacillus bulgaricus***	***Lactococcus lactis***	***Streptococcus thermophilus***	***St. thermophilus* and *L. bulgaricus* 1:1**
Total coliform plate count	<10	<10	<10	<10	<10
Yeasts and molds plate count	<10	<10	<10	<10	<10
Staph. aureus Detection	N.D *	N.D	N.D	N.D	N.D
Bacillus cereus Detection	N.D	N.D	N.D	N.D	N.D
E.coli O157 : H7 Detection	N.D	N.D	N.D	N.D	N.D
Salmonella spp. Detection	N.D	N.D	N.D	N.D	N.D
Listeria monocytogenes Detection	N.D	N.D	N.D	N.D	N.D

N.D* = Not detected in 25 ml of sample.

molds and total coliform counts are shown in Table 1. The results of this work showed that final products of fermented camel milk prepared in the lab by using five starter cultures had no *Salmonella spp*., *S. aureus*, *L. monocytogenes, E. coli O157:H7* or *B. cereus,* while the total coliform, yeast and mold counts were less than 10 cfu per ml. The absence of the pathogens was mostly probabaly due to the correct pasteurization process, strict hygiene conditions during preparation and to the use of starter which reduced the pH of the products. This finding is in agreement with the report of Puzyrevskaya, et al. (2000) who documented that fermented camel milk contains lactic bacteria which reinforced the antimicrobial activities against pathogenic agents.

According to Guizani et al. (2001), traditional fermented laban samples collected from small-scale produce in Sultanate of Oman showed considerable number of yeasts and molds, coliforms and fecal coliform while they were not detected in the commercial laban samples. Similarly Al-Tahiri.(2005) reported that the traditional fermented milk products in Jordan showed a high viable count of total coliform, yeast and molds and *S. aureus* while the dairy products produced by modern dairies showed a very high quality of microbial standard with a very delicate flavor. On the other hand, the results obtained from microbial analysis of Moroccan traditional fermented dairy products like Lben and Jben showed high number of coliforms, enterococci and pathogens such as *Salmonella spp., Yersinia enterocolitica, L. monocytogenes* and *S. aureus* (Hamama and Bayi, 1991).

Similarly, the results obtained from the microbial analysis of *nono* and *wara* (local traditional fermented dairy products widely consumed in many African countries) show that both products were contaminated with microorganisms of public health concern (Uzeh et al., 2006). Savadogo et al. (2004) also investigated *Fulani* traditional fermented milk in Burkina Faso and found little numbers of *Salmonella, Shigella* species and high numbers of coliforms in some samples. All these results can be explained by the fact that the methods of production of the various traditional foods are usually primitive compared to modern ways of food preparation (Dirar, 1997; Isono et al., 1994) and the major risk enhancing factors are the use of contaminated raw materials, lack of pasteurization, use of poorly controlled natural fermentations, inadequate storage and maturation conditions (Nout, 1994).

Biochemical analysis

Changes in the Total acidity and pH

Figures 2 and 3 show the changes in pH and total titratable acidity (expressed as percent lactic acid) of the camel milk inoculated by five starter cultures incubated at 43ºC for 6 h. The amount of lactic acid produced increased with concomitant drop in pH with an increase in fermentation time. The initial pH of the inoculated camel milk for the 5 cultures at the start of fermentation was 6.25 (*L. acidophilus*), 6.22 (*L. bulgaricus*), 6.24 (*Lactococcus lactis*), 6.22 (*St. thermophilus*) and 6.21 (combination strains of *L. bulgaricus* and *St. thermophilus*, 1:1), whereas at the end of fermentation (6 h) the pH decreased to 5.00, 4.60, 5.35, 5.00 and 4.35 while the total acidity increased from initial values of 0.17,0.18,0.18,0.17and 0.17 to 0.44, 0.78, 0.35, 0.48 and 0.83 for *L. acidophilus, L. bulgaricus, L. lactis*, *St. thermophilus* and combination of *L. bulgaricus* and *St. thermophilus* (1:1) cultures, respectively. The results indicated that the pH of camel milk fermented by *L. bulgaricus* was lower than those fermented by other single cultures, whereas the combination of *L. bulgaricus* CH2 plus *St. thermophilus 37* (1:1) gave lower pH and higher acidity compared to the pure single starter cultures. These results are in agreement with those observed by Rajagopal and Sandine. (1990) and Carrasco et al. (2005), who reported that the *St. thermophilus* cultures have higher pH than the *L. bulgaricus* cultures and the pH for mixed cultures was much lower than those for the pure cultures.

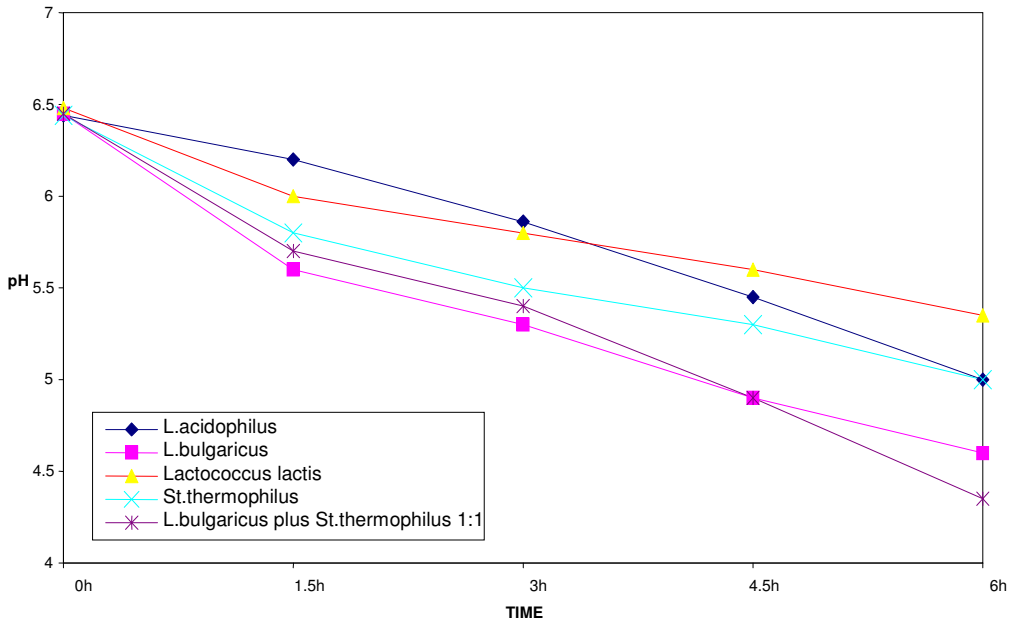

Figure 2. PH changes during fermentation of camel milk at 43°C for 6 h.

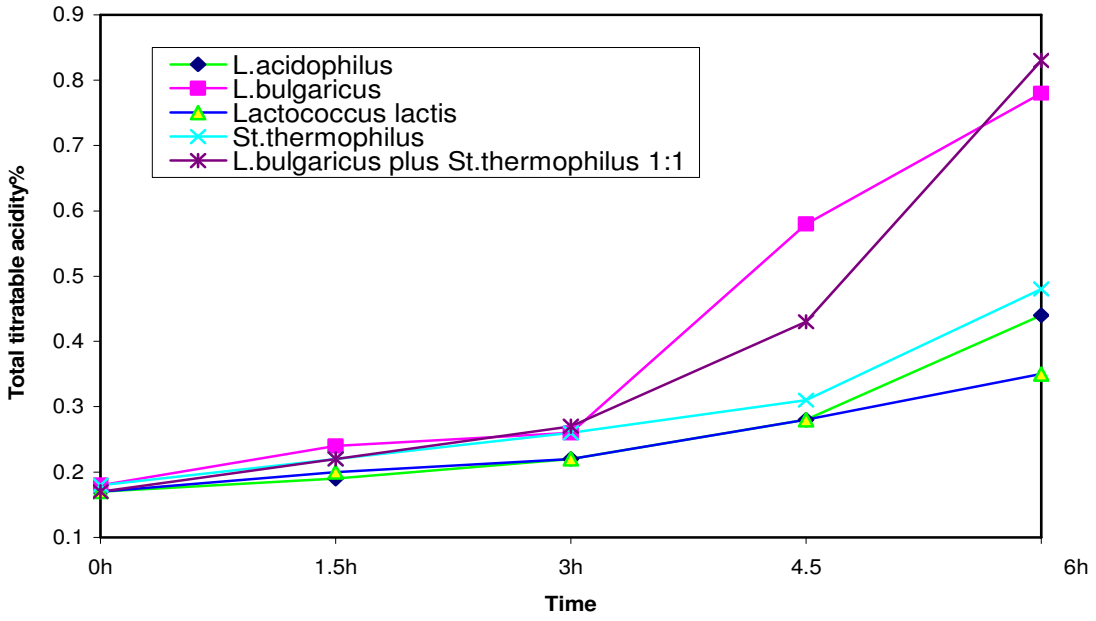

Figure 3. Changes in total titratable acidity (expressed as lactic acid %) during fermentation of camel milk for at 43°C for 6 h.

Similarly Abu-Tarboush (1996) studied the behavior of different strains of commercial cultures in whole camel milk incubated at 42ºC for 4 h and found that the final pH of *L. bulgaricus CH2* was lower than that of all single streptococcal and lactobacilli strains and the combination *L. bulgaricus 12* with the other strains of *St. thermophilus* resulted in lower pH than with either single culture alone. The present results revealed that the pH of the fermented camel milk by the five starter cultures ranged from 5.35 to 4.35 which is similar to that reported in suusac (pH 4.30), a Kenyan traditional fermented camel milk product (Lore et al., (2005), but higher than those in garris (pH 3.25 - 3.40) a Sudanese traditional fermented camel milk (Mirgani, 1994). The amount of lactic acid obtained from camel milk fermented with *L. acidophilus* at 43ºC for 6 h were in agreement with those

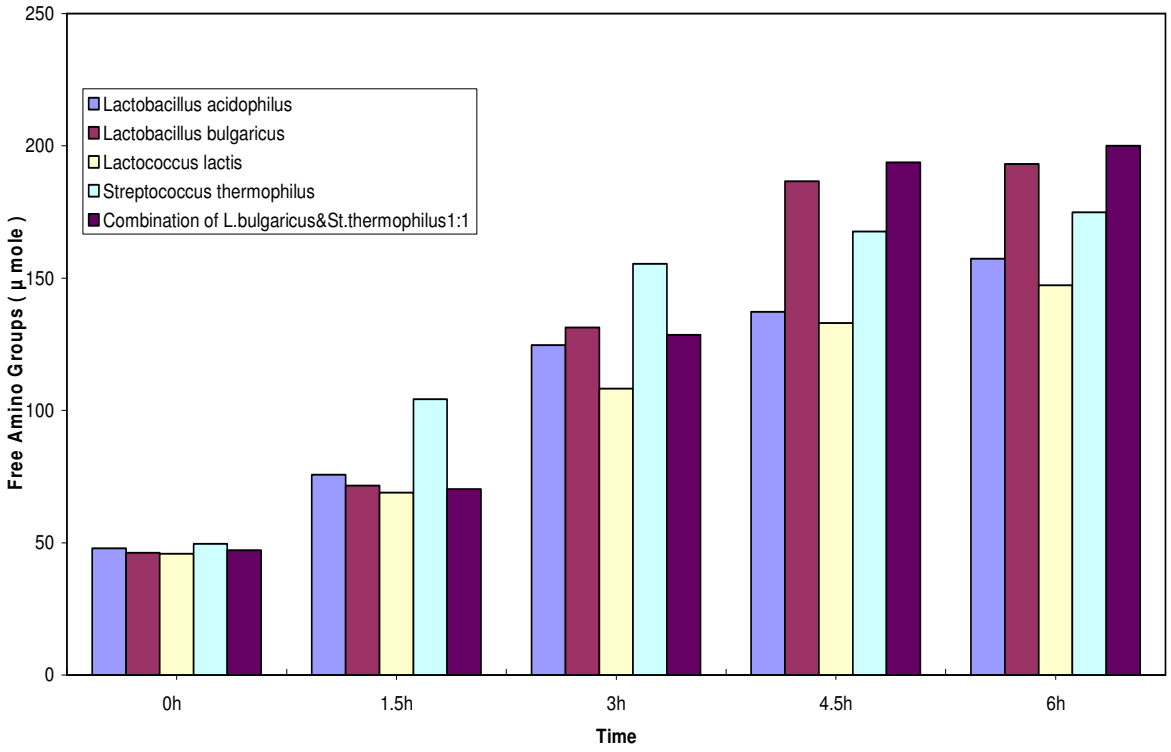

Figure 4. Changes in proteolytic activities of the starter culture strains during fermentation of camel milk for 6 h at 43°C.

found in the acidophilus milk made from camel milk by Abu-Tarboush. (1994).

Proteolytic activities of starter cultures in camel milk

The changes in proteolytic activities of *L. acidophilus, L. bulgaricus, L. lactis*, *St. thermophilus* and mixed cultures of *L. bulgaricus* and *St. thermophilus* (1:1) during fermentation of camel milk at 43ºC for 6 h are presented in Figure 4. The amount of free amino groups (FAG) released after 1.5 h of fermentation were 75.65, 71.62, 68.92, 104.25 and 70.30 µg/ml for the five starter cultures respectively. The above results indicated that the amount of FAG released by *St. thermophilus* (104.25 µg/ml) was the highest compared to the other starter cultures. Moreover, when the fermentation time was increased up to 6 h, there was a subsequent increase in the FAG amount to 157.31, 193.14, 147.37, 174.9 and 199.98 for the five starter cultures resp. When comparing the different treatments, the amount of FAG released after 6 h was highest in the mixed starter cultures of *L. bulgaricus* and *St. thermophilus* (1:1) than in the corresponding single cultures. In general these results showed that *Lactobacillus* strains had higher proteolytic activity than the *Lactococcus lactis* strain.

This observation agreed with the findings of Sasaki et al. (1995) who reported that *Lactobacillus* strains had a higher proteolytic activity than the *Lactococcus* strains. Similarly, Rajagopal and Sandine (1990), reported that the lactobacilli strains were highly proteolytic than streptococci and the mixed cultures always liberated more tyrosine in cow skim milk than the sum of the corresponding single cultures. Also Rao *et al*., 1982 found that fermentation of milk by various lactic acid bacteria increased the free amino acids content and that *L. bulgaricus* was found to be the most proteolytic of all organisms used. Our data differed from that reported by Abu-Tarboush (1996) who found that the amount of FAG released by mixed cultures in camel milk incubated at 42ºC for 4 h was almost the same as that produced by any of the corresponding single cultures except for *L.bulgaricus lb12*. On the other hand, Shihata and Shah (2000) and Carrasco et al. (2005) independently reported that the yogurt bacteria (*S. thermophilus* and *L. delbrueckii ssp. bulgaricus*) appeared to be highly proteolytic as compared to the probiotic bacteria (*L. acidophilus* and *Bifidobacterium spp*) and released higher amount of free amino acids.

Sensory evaluation of fermented camel milk products

Five samples of camel milk fermented for 6h at 43ºC by selected starter cultures were prepared and sensory evaluated by 10 untrained panelists for color, smell, consistency, taste and overall acceptability. The mean values of sensory evaluation scores are summarized in

Table 2. Summary of sensory evaluation scores of camel milk fermented for 6h at 43ºC.

Attribute	Camel milk fermented for 6 h at 43ºC by selected starter cultures				
	Lactobacillus acidophilus	***Lactobacillus bulgaricus***	***Lactococcus lactis***	***Streptococcus thermophilus***	**Yogurt Culture ***
Color	8.1±0.78[a]	7.9±0.74[a]	8.0±0.82[a]	8.0±0.82[a]	7.9±0.87[a]
Smell	6.0±0.79[c]	6.4±0.67[b]	5.1±0.84[d]	6.2±0.95[c]	7.5±0.57[a]
Consistency	3.2±0.88[c]	3.6±0.97[b]	2.8±0.97[d]	3.3± 0.97[c]	4.3±0.97[a]
Taste	6.6±0.67[c]	.3±0.74[b]	5.5±0.71[d]	6.4±0.70[c]	7.5±0.79[a]
Overall acceptability	7.2±0.67[b]	7.1±0.74[b]	5.5±0.71[d]	6.4±0.70[c]	7.6±0.79[a]

- Values are means ± SD.
- Means not sharing a common following letter in a raw are significantly different at $p < 0.05$.
- A 9-point hedonic rating scale (9 = excellent ; 1 = extremely poor).

* Combination of *St .thermophilus and L. bulgaricus* 1:1.

Table 2.The mean scores value for color of the all fermented samples ranged from 7.9 to 8.1 (good). The results showed that there were no significant differences ($p > 0.05$) in color of the five fermented products. The mean score for smell of camel milk fermented by yogurt culture (*L. bulgaricus* and *St. thermophilus* (1:1) was significantly higher ($p < 0.05$) than mean scores for other fermented milk products by other starter cultures, indicating that camel milk fermented by yogurt culture (7.5) was the most acceptable followed by those fermented by *L. bulgaricus* (6.4), *St. thermophilus* (6.2) and *L. acidophilus* (6.0) while the least acceptable was that fermented by *Lactococcus lactis* (5.1).

In general, the panelists gave lower sensory scores for consistency for all fermented camel milk but that one fermented by yogurt culture was slightly better in consistency score (4.3) than those fermented by other starter cultures. The panelists preferred fermented camel milk made by yogurt starter culture followed by *L. bulgaricus, L. acidophilus*, *St. thermophilus* and *L. lactis*. The overall acceptability scores of the sensory evaluation revealed that the camel milk fermented by yogurt starter culture was the most accepted, while that fermented by *L. lactis* was the least. Camel milk fermented by yogurt culture had significantly ($P < 0.05$) higher rating for smell, consistency, taste and acceptability compared with other cultures. However, the consistency of all fermented camel milk products was watery and showed a fragile and heterogeneous structure. These finding agree with those of Abou-Tarboush (1994) who reported that acidophilus milk made from camel milk was watery and precipitated in the form of flocs. Similarly, Attia et al. (2001) observed that the fermentation of camel milk by starter culture did not reveal curd formation but indicated a fragile and heterogenous structure. Fermentation of camel and cow milk by lactic acid bacteria indicated also that the cultures were less active in camel milk than cow milk and the camel milk failed to reach a gel-like structure after 18h incubation (Gran et al., 1991). The author attributed that to the presence of growth inhibitors in camel milk. Farah. (1990), reported that the Susa traditional fermented camel can be improved by using selective mesophilic lactic acid culture.

Conclusion

The data indicate that certain microbial and biochemical changes occur during fermentation of camel milk. The results showed that microorganisms differed in growth, acid production and proteolytic activities. In general mixed yogurt culture showed superior growth, acid production and proteolytic activity than single starter cultures. Sensory results also indicated that mixed yogurt culture produced acceptable fermented camel milk. Additional work is needed on the consistency of fermented product.

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