Biochemical Changes in Camel Milk Fermented by Bacterial Starter Cultures

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Abstract

Fresh whole camel milk from *Camelus dromedarius* was obtained from a private herd and investigated preliminarily for its chemical composition, fatty acids profile, amino acids composition, vitamins contents (vitamin C, riboflavin and thiamine) and microbial counts.

The microbiological and biochemical changes that occur during the fermentation of camel milk inoculated with each of five selected lactic acid cultures at 43ºC for 6h, were studied as well as the sensory evaluation of the products. The five cultures were: *Streptococcus thermophilus 37*, *Lactobacillus delbrueckii sub sp. bulgaricus CH2, Lactococcus lactis, Lactobacillus acidophilus* and mixed yogurt culture (*S. thermophilus* and *L. bulgaricus* 1**:**1).

The total viable counts of the starter cultures throughout fermentation period (6h) showed that the *L. bulgaricus* was always more numerous than the other single starter cultures while *Lactococcus lactis* was least numerous. The combination of *L. bulgaricus* and *S. thermophilus* showed more counts than the single strains. The microbiological investigation of the fermented camel milk products conformed with the microbial standard.

The biochemical changes in the camel milk inoculated with each of the five cultures at 43ºC for 6h indicated that *L.bulgaricus* gave lowest pH and released high amount of free amino acids among all the cultures except the combined one.

The chemical composition of the fermented camel milk indicated that no significant differences were observed in total solids of fermented camel milk after 6h of fermentation, except for that fermented by *L. acidophilus* was higher compared to raw milk. Results of this research work indicated that a majority of the fatty acids of raw milk were not affected by fermentation except the levels of myristic acid, oleic acid and palmitic acid that increased while palmitoleic acid and arachidic acid were decreased. The amino acids content was increased slightly due to fermentation compared to the unfermented milk. The fermented camel milk products showed significant decreases in vitamin C and insignificant decreases in riboflavin and thiamine contents compared to unfermented pasteurized milk. Sugar analysis of fermented milk showed decrease in lactose content and increase in glucose and galactose. In fermented camel milk products, lactic acid was found in the highest concentration, followed by formic acid and acetic acids.

The results of the sensory evaluation study indicated that the camel milk fermented by yogurt culture was the most accepted while

that one fermented by *Lactococcus lactis* was the least. However, the consistency of all camel milk products was watery and showed a fragile, poor structure (poor scores).

خلاصة الأطروحة

تم الحصول على حليب الإبل الخام المستخدم في هذه الدراسة من أحد القطعان الخاصة حيث تم إجراء تحليل مبدئي للتركيب الكيميائي والأحماض الدهنية والأحماض الأمينية والفيتامينات (فيتامين ج - ب2 - ب1) والعد الميكروبي.

تم تلقيح الحليب بواسطة بادئات مختارة من بكتيريا حمض اللاكتيك وتحضينه على 43 درجة مئوية لمدة 6 ساعات ثم أجريت دراسة ميكروبيولوجية وكيميائية للتغيرات التي تحدث أثناء عملية التخمر كما تم أيضاً إجراء التقييم الحسي للمنتج النهائي. وشملت هذه الميكروبات استربتوكوس ثيرموفيلس، لاكتوباسيلس بلقاريكس، استربتوكوكس لاكتيس، لاكتوباسيلس اسيدوفيلس وخليط من بادئ الزبادي (استربتوكوس ثيرموفيلس ولاكتوباسيلس بلقاريكس 1:1)

أظهر تقدير العد الكلي بالأطباق لأنواع المزارع الميكروبية أن أعداد بكتيريا اللاكتوباسيلس بلقاريكس كان الأكثر خلال عملية التخمر مقارنة بالبادئات الأخرى المستخدمة في صورة فردية بينما اللاكتوكوكس لاكتيس كان الأقل0 خليط بادئ الزبادي (بكتيريا اللاكتوباسيلس بلقاريكس وستريبتوكوكس ثيرموفيلس 1:1) كان الأكثر عددا في نهاية التخمر عن اعداد المزارع المستخدمة بصورة فردية. الاختبارات الميكروبيولوجية التي أجريت على المنتج النهائي دلت على أن المنتج ذا جودة ممتازة ميكروبيولوجياً.

أظهرت التغيرات الكيموحيوية في حليب الإبل الملقح بواسطة المزارع الخمسة على درجة 43 مئوية لمدة 6 ساعات أن بكتيريا اللاكتوباسيلس بلقاريكس أعطى أقل أس هيدروجيني وأعلى نسبة من الأحماض الأمينية الحرة عند نهاية التخمر مقارنة بالميكروبات الأخرى ماعدا خليط بادئ الزبادي.

أظهرت دراسة التركيب الكيميائي لحليب الإبل المخمر انه لا توجد فروق معنوية في الجوامد الكلية للحليب المخمر بعد 6 ساعات بواسطة البادئات المختلفة عدا المخمر بواسطة بادئ اللاكتوباسيلاس اسيدوفلس كان أعلى مقارنة بالحليب الخام. معظم الأحماض الدهنية للحليب الخام لم تتأثر بعملية التخمر عدا نسبة تركيز حمض الميرستيك، حمض الاوليك وحمض البالمتيك زادت بينما نسبة تركيز حمض البالمتيوليك والاراكديك نقصت. قيمة الأحماض الأمينية ازدادت قليلاً نتيجة لعملية التخمر. لوحظ انخفاض معنوي في محتوى فيتامين ج وانخفاض طفيف غير معنوي في فيتامين ب$_2$ و ب$_1$ وأيضاً وجد انخفاض في تركيز اللاكتوز وزيادة في تركيز الجلوكوز والجلاكتوز في الحليب المخمر عن ما هو في الحليب المبستر غير المخمر. الأحماض العضوية الرئيسية التي وجدت في العينات المخمرة كانت حمض اللاكتيك الأعلى تركيزاً ويليه الفورميك والخليك.

أظهرت نتائج التقييم الحسي للعينات المخمرة أن الحليب المخمر بواسطة بادئ الزبادي كان الأفضل من حيث اللون والرائحة والطعم والقبول العام بينما الأقل قبولاً كان الحليب المخمر بواسطة بكتيريا اللاكتوكوكس لاكتيس ولكن كل العينات كانت مائية و فقيرة في القوام.

1. Introduction

Camels belong to the family *Camelidae* and the sub-order *tylopoda*. The *Tylopoda* themselves belong to the order *Artiodactyla* or clove-footed animals. The *camelidae* originated in North America where their earliest fossil remains have been found. It crossed the ancient land bridge which is now the Bering Straits into Asia and the Middle East and Africa before 10 million years ago. Some of the camels migrated to the deserts and semi-deserts of northern Africa and the Middle East (Simpson, 1945; Zeuner, 1963).

The population of camels in the world is about 19 million of which 14.5 million are found in Africa and 4.9 million in Asia. Of this estimated world population, 17 million are believed to be one-humped camels and 1.9 million two-humped. Sixty percent of the camel population is concentrated in the four North East African countries of Somalia, Sudan, Kenya and Ethiopia (Table 1, FAO, 1990, 1978). Milk yield estimates from many sources indicate that the camel is potentially a better milker than many African Zebu cows under the same environmental conditions. While the daily milk yield of a Zebu cow is 0.5 - 1.5 Kg (Kiwuwa, 1973), Speneer (1973) estimated that one Rendille camel in Kenya produced as much milk as did four cows.

Table (1). World camel (dromedaries) population (millions)

Country	1978	1990
North East Africa		
Somalia	5.4	6.85
Sudan	2.9	2.8
Ethiopia	1.0	1.08
Kenya	0.6	0.8
Total	9.9	11.53
West Africa		
Mauritania	0.7	0.82
Chad	0.4	0.54
Niger	0.4	0.42
Mali	0.2	0.24
Nigeria	--	0.18
Senegal	0.1	0.15
Total	1.8	2.35
North Africa		
Tunisia	0.2	0.18
Algeria	0.1	0.13
Egypt	0.1	0.19
Libya	0.1	0.19
Morocco	0.02	0.04
Total	0.5	0.73
Asia		
India	1.2	1.45
Pakistan	0.8	0.99
Afghanistan	0.3	0.26
Iraq	0.2	0.05
Saudi Arabia	0.1	0.4
Iran	0.03	0.02
Total	2.7	3.17

Source: FAO production Yearbook No.32 (1978) and No. 44 (1990).

Sudan has the second largest number of camels in Africa. They belong to the one-humped dromedary kind, which originally reached the country from Arabia and are only found in the northern states and mostly owned by the nomads who inhabit the acacia desert zone. The population of camels (*Camelus dromedarius*) in Sudan was estimated to be 2.7 million distributed around the country as follows: 3%, 2%, 6.4%, 45.6%, 16.4% and 23.6% in Northern, Khartoum, Central, Kordofan , Darfur and Eastern States, respectively (Tibin,1988).

The Ministry of Animal Resources (1996) gave an estimate of annual milk production in Sudan of about 7.58 million tons of which 5 million (66%) are cow's milk. Goat's milk made 1.9 million tons, sheep's 0.65 million tons and camel's milk only 0.033 million tons. 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. El-Amin (1979) noted that the average daily milk yield of camels in Sudan was found to be 5-10 Kg. On the other hand, Hassan (1968) found that the Zebu cow of the southern Sudan gave an average daily yield of only 0.5 kg of milk, whereas the same cow when better fed at the Juba Dairy Farm gave a yield of 2.3 kg daily.

Fermentation of milk is a very ancient practice of man; the majority of fermented milk is made from cow milk, followed by sheep, goat and camel milk. Through the years, the fermentation process was improved by saving some of the fermented product and using it to start the next batch. In many cases the bacteria found in modern starter cultures of today include those bacteria that predominated in the original traditional fermented foods. Modern pure cultures have been developed by isolating and using those same bacteria to manufacture the product under sanitary and controlled conditions to ensure that the desired bioconversion occurs in producing the food. In order to reduce the number of undesirable microorganisms in the fermented products, milk is exposed to treatments such as heat prior to adding the starter cultures.

Dirar (1993) estimated that 50-60% of the annual milk production in Sudan was turned into dairy products. He divided the fermented dairy products of the Sudan into two major groups: the truly indigenous which include rob, gariss, biruni and mish, and the quasi - indigenous which include zabadi and jibna beida. On the other hand, a report by the Arab Organization for Agriculture Development (AOAD, 1983) estimated the milk turned into dairy products in Sudan

to be 65% of the annual total. Rob makes about 90% of all fermented milk products, as an offhand estimate.

There is an increasing trend all over the world for the consumption of fermented milk products due to the fact that they have a unique flavor, desirable texture, and are regarded as safe. Moreover, they contain an excellent nutritional profile and have an image of being natural .The nutritional value of a food is dependent not only upon its nutritional content, but also upon the availability, digestibility and assimilability of the nutrients. Recent scientific and technological advances showed 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). The natural antibiotics, generally referred to as bacteriocins, produced by the cultures inhibit the pathogenic bacteria in the gut. For example, the antibiotic acidophilin, produced by *Lactobacillus acidophilus* containing foods has been shown to inhibit 50% of 27 different disease-causing bacteria. Children with *Salmonella* poisoning and *Shigella* infections were cleared of all symptoms using acidophilus milk (Shahani *et al*., 1976).

There is good incentive to expand the range and quality of traditional fermented products; attention is focused on the need for pure cultures of lactic acid bacteria that could be used successfully in the dairy industry. Lactic acid bacteria can ferment lactose in a variety of ways; there are two main types of lactic acid bacteria: homofermentative bacteria that mainly produce lactic acid and heterofermentative types that ferment glucose to produce carbon dioxide, alcohol and in some cases, acetic acid.

The objectives of this study were as follows:

1- To study the microbiological and biochemical changes in the camel milk during the fermentation period.

2- To develop fermented camel milk by using selected pure cultures.

3- To test the acceptability of the product in comparison with the traditional fermented camel milk in Sudan.

4- To reduce preparation times and improve yields of product.

5- To control the level of hygiene and sanitation.

2. Literature Review

2.1. Chemical composition of raw camel milk

Milk is the lacteal secretion, practically free from colostrum, obtained by the complete milking of one, or more, healthy animal, and which contains not less than 8.25 percent milk total solids (not fat), and not less than 3.25 percent milk fat; the main constituents of milk are water, fat, proteins (such as casein and albumin), lactose (milk sugar), and ash (Johnson 1974).

Camel milk is generally opaque-white and has a sweet and sharp taste, but sometimes can also be salty. The changes in taste are caused by the type of fodder and availability of drinking water. One of the important factors that affect camel milk composition is water. Yagil and Etzion (1980) examined the effects of restricting drinking water on camel milk, while the diet remained unchanged throughout the year; great changes in water content of milk were found.

The colostrum of camels is white and slightly diluted and as compared to the colostrum of the cow (Rao *et al*., 1970; Yagil and Etzion, 1980).

Sestuzheva (1958) studied ten Kazakhstan camels, and reported that the first colostrum obtained after 3 hours post partum contained

on average 30.4% total solids, 0.2% fat, 19.4% protein, 7.2% lactose and 3.8% mineral. During the first 2 days of lactation, total solids fell to 18.4%, because of the decline in total proteins and minerals to 3.6%, and 0.1% respectively; lactose level remained practically unchanged while the fat content increased to 5.8%. The composition then remained constant for ten days. In a similar study, Abu-Lehia *et al*. (1989) examined the colostrums of ten Saudi camels during their first season of lactation up to 10 days post partum. At parturition, the contents of total solids, fat, protein, lactose and minerals were 20.5, 0.20, 13.0, 2.7 and 1%, respectively. After 3d lactation total solids decreased to 13.6%, protein to 4.7% and minerals to 0.8%. However, the fat content rose to 1.5% and lactose to 4.4%. Recently, Merin *et al*. (2001) studied chemical composition of camel's colostrum and milk from parturition until 5.5 month and found that camel's colostrum was poorer in fat in the first 4 days post partum than cow's colostrum.

The chemical composition of camel milk has been studied in various parts of the world (Ohri and Joshi, 1961; El Bahay, 1962; Sohail, 1983; Abu-Lehia, 1987). The general composition of camels milk varies in various parts of the world with a range of 3.07-5.5% fat,

3.5-4.5% protein, 3.4-5.6% lactose, 0.7-.95% ash, and 85.87-90% water (Anon, 1982).

The proximate analysis of camel milk in Saudi Arabia studied by El-Amin and Wilcox (1992) indicated 3.15% fat, 2.81% protein, 4.16% lactose, 10.95% T.S., 0.83% ash and 88.33% water. In a similar study, Mehaia *et al*. (1995) studied the chemical composition of camel milk from three ecotype camels (Majaheim, Wadah and Hamra). They found that pH, percentage acidity, total solids, fat, protein, lactose and ash for Majaheim were 6.63, 0.144, 11.35, 3.22, 2.91,4.43 and 0.79%, for Wadah they were 6.65, 0.41, 10.07, 2.46, 2.36, 4.44, 0.81%, and for Hamra they were 6.65, 0.137, 10.63, 2.85, 2,52, 4.46 and 0.80%, respectively. In another study, Sawaya *et al*. (1984a) gave the following proximate composition of camel milk: T.S.11.7%, protein 3.0%, fat 3.6%, lactose 4.4%, ash 0.8%, 0.13% acidity and a pH of 6.5.

In Sudan, Mirghani (1994) found that chemical composition of camel's milk was, 4.7% lactose, 3.0% fat, 3.8% protein, 0.72% ash, 88.5% moisture, 0.17% acidity (as lactic acid) and a pH of 6.5. Similar results were observed by Abdel-Rahim, (1987) and Auru, (1987).

In India, camel milk collected from the surrounding area of Udaipur city was found to contain 12.1% T.S., 3.74% fat, 2.86% protein 4.62% lactose and 0.78% ash, whereas the values for specific gravity, viscosity, acidity, and pH were 1.031, 1.62 centipoises, 0.098% and 6.69, respectively (Sankhla *et al*., 2000). According to Gnan and Sheriha (1986), the milk of the Libyan camels contains 3.30-3.66% fat, 3.3-3.53% protein, 5.61-4.16% lactose, 87.02-87.3% moisture, 0.82-1.15% ash, and a pH range of 6.82-6.20 and the average fatty acid composition contained high concentrations of linoleic and polyunsaturated acid.

The pH of camel milk is between 6.5-5.7, which is similar to the pH of sheep's milk (Shalash, 1979). When camel milk is left to stand, the acidity rapidly increases; the lactic acid content increases from 0.03% after standing for 2 hours to 0.14% after 6 hours (Ohri and Joshi, 1961). In the Sudan, the lactic acid percentage of fresh camel milk had a range of 0.1% to 0.22% and the pH ranged from 6.0 to 6.5 (Adam, 1987; Auru, 1987; Farid, 1987).

The first data of fatty acid composition in camel milk were published by Dhingra (1934), who examined the milk fat of Indian camels using old techniques of fractional distillation. Further data on

the subject were provided by Hagrass *et al*. (1987), Abu-Lehia (1989), Farah *et al*. (1989), Farag and Kebary (1992), Mohamed and Hjort (1993), and recently Gorban and Izzeldin (2001). They agree that the general pattern of the camel milk fatty acids indicates that short-chain fatty acids C4-C12 are present in very small amounts compared with cows milk fat, but the concentrations of C14:0, C16:0 and C18:0 are relatively high. They also stated that there is a high content of polyunsaturated fatty acids in camel colostrums and milk.

The total protein of camel milk is similar to that of cow milk; however, camel milk caseins and their fractions were found to be poor in crude protein when compared with cow milk (Pant and Chandra, 1980). In another study, Beg *et al*. (1986) found that camel milk protein contained a new kind of protein, a fraction of the Beta-casein, and it had an extremely low capa-casein content which is probably behind the fact that the milk does not curdle easily. Other investigators like Larsson and Mohamed (1986) found similar results.

The amino acids composition determined in 15 samples of camel milk in Sudan and compared with cow milk casein, showed more proline and threonine in camel milk casein, but less alanine, arginine, glycine, histidine and serine. (Holler and Hassan, (1965). In

contrast, the data reported on amino acid composition by Sawaya *et al*. (1984a); Farah and Ruegg (1989) and Mehaia and Al-kanhal (1989) generally showed that the amino acid content of camel milk protein was similar to that of cow milk.

Camel's milk considerably contains less of vitamin A and riboflavin than cow's milk, while the level of vitamin C was on average 3 times higher than that of cow's milk (Farah *et al*., 1992). Ahmed (1990) analyzed 364 samples of camel milk by HPLC and found similar results. Sawaya *et al*. (1984a) analyzed eleven camel milk samples in Saudi Arabia, and found that the vitamin C content was 23.7, thiamin content was 0.33 and the riboflavin content was 0.416 mg/kg. Mehaia (1994) found vitamin C and riboflavin content in camel milk were 24.9 and 0.56 mg/kg, respectively. The author concluded that the levels of vitamin C and riboflavin in raw camel milk were higher than cow's milk. The author reported decreases in vitamin C of about 27, 41, 53, and 67% for samples of raw camel milk heated at 63, 80, 90, and 100C° for 30 min, respectively .However heat treatment caused a negligible amount of destruction (0-7%) of the riboflavin content.

2.2. Antibacterial activity of camel milk

The antibacterial activity of camel milk protective proteins was studied by many workers like El Agamy, *et al*. (1992) and El Agamy. (1994). They concluded that the camel milk contained a variety of protective proteins that contributed to bacterial growth inhibition (lysozyme, lactoferrin, lactoperoxidase and immunoglobulin). The activity of these protective proteins was assayed against *Lactococcus lactis subsp. cremoris, Escherichia coli, Staphylococcus aureus* and *Salmonella typhimurium*. They found that antibacterial activity of camel milk lysozyme was similar to that of egg white lysozyme but different from the bovine lysozyme. Camel milk lactoperoxidase was bacteriostatic against the Gram positive strains and bactericidal against Gram-negative strains but immunoglobulins had little effect against the bacteria. In other study Kappeler *et al*. (1999) underlined that the higher amounts of lactoferrin in camel milk are of advantage for natural preservation of the milk in arid regions, where technology for milk preservation is often not available.

Barbour, *et al*. (1984) studied the ability of camel milk to inhibit growth of pathogenic bacteria and its relation to whey lysozyme. Results indicated that 20 out of 200 samples collected from

individual camels inhibited growth of one or more of six pathogenic test organisms.

2.3. Bacterial count in raw camel milk

Many investigators around the world studied the keeping quality of fresh camel milk. In Saudi Arabia, Al-Mohizea (1986) examined 29 samples of fresh camel milk collected from Riyadh shops. He found that the mean counts were $3.3x10^5$ cfu/ml (range $1.7x10^2$-$1.5x10^2$ cfu/ml) total aerobes count, $2.2x10^3$ cfu/ml (1-$8.4x10^3$), total staphylococci and $1.4x10^4$ cfu/ml (6x10-$7.3x10^4$) total coliforms in the raw camel milk; he underlined the potential of health hazard to the traditional consumers who drink camel's raw milk. Adam (1987) and Auru (1987) independently noted that the total viable count of fresh camel milk ranged from $9.2x10^2$ to $4.2x10^7$ cfu/ml, while Farid (1987) showed a range of $8.3x10^3$ to $2.4x10^4$. In Egypt, Mustafa *et al*. (2000) analyzed 50 raw camels milk. Results indicated that aerobic plate count, enterococci and total yeast and molds were $4.3x10^4$, $2.9x10^2$ and $4.5x10^2$ cfu/ml, respectively.

The bacteriological quality of raw camel milk in Ethiopia was assessed by Semereab and Moeller (2001). They found that the milk sample directly from the udder contained mainly staphylococci and

streptococci while samples taken from milking bowls were additionally contaminated with enterobacteria.

The bacteriological quality of raw milk may be considerably improved by strictly hygienic methods of milk production (Thomas and Druce, 1971; Chatelin and Richard, 1981; Nout, 1994).

2.4. Traditional fermented milks

2.4.1 Microorganisms involved in fermentation

Fermentation is generally defined as a chemical change that is brought in the base food due to the action of inoculated cultures and the enzymes they produce. One of the most ancient practices of man is the acid fermentation of milk to obtain products with particular characteristics of flavor, smell and consistency which can be kept over a long period; that fermentation is one of the methods of food preservation. Over the years this method has evolved into a sophisticated art. Milk can be fermented by bacteria, yeasts, and mold to produce a variety of products such as yogurt, cheese, sour cream, and buttermilk. Modification of milk by microorganisms affects both the physiochemical properties and economic value of milk; the physiochemical changes are manifested in such properties as flavor, texture, and nutritive value. The economic value of milk is enhanced

by the increased storage life of the products. There are several fermented milk products that are in use in different countries with different names, and there are some common microorganisms that are generally used in developing these fermented foods but one also finds some differences in the culture composition. Kosikowski (1978) classifies fermented milk into 4 types: (1) acid/alcohol such as kefir and koumiss; (2) high acid such as Bulgarian sour milk; (3) medium acid such as acidophilus milk and yogurt; and low acid such as cultured buttermilk and cultured cream.

Abdelgadir *et al*. (1998) reviewed the traditional fermented dairy products of Sudan (vitamins in fermented milk, health claims of fermented milk, lactic acid involved in fermentation) and reported that the indigenous dairy products include rob, samin, gariss, biruni, mish, zabadi and jibna-beida. In a similar study Mutukumira *et al*. (1995) reviewed traditional fermented milk in some sub-Saharan countries with particular focus on Zimbabwe fermented milk products. Many workers from different countries reported about the microorganisms associated with the traditional fermented milk. The first work on the microbiology of fermented milk in Sudan was probably that work carried out by Dirar (1975) who reported that milk would sour by

Enterobacter aerogenes, which produced a frothy product, or by *Lactococcus spp.,* which produced a smooth-set product. So, both coliform bacteria and lactic acid bacteria are involved in the souring of milk, especially in the hot summer.

Laban (traditional fermented milk in Lebanon) had a titratable acidity of about 1.0%, a pH of 4.25, an ethanol content of 1.25% and contained 4.2-µg per ml acetaldehyde. Five microorganisms, classified as *Streptococcus thermophilus, Lactobacillus acidophilus, Leuconostoc lactis, Kluyveromyces fragilis* and *Saccharomyces cerevisiae* were responsible for the fermentation (Baroudi and Collins, 1976). The microflora of traditional Omani laban consisted predominantly of mesophilic *Lactococcus* and homofermentative lactobacilli (1.3×10^{8} and 2.4×10^{6} cfu/ml, respectively) with high numbers of yeast, coliforms and fecal coliforms (Guizani and Al-Ramadani, 1999). In Egypt, investigation of laban rayeb showed a high level of enteric bacterial contaminants with *Enterococcus* as the predominant species (Khalafalla *et al*., 1988). Recently Uzeh *et al*. (2006) studied the microbiological quality of two fermented dairy products (*Nono* and *Wara* – local dairy products in Nigeria) and reported that total plate count was 3.6×10^{8} and $4.6\times10^{8,}$ the coliform

count was 4.3×10^7 and 2.4×10^7 while fungal count was 1.3×10^6 and 1.3×10^7 cfu/ml for nono and wara, respectively.

Rob (Sudanese traditional fermented milk) was investigated by many workers. Saeed (1981) found that the major microbial group of rob included lactobacilli, lactococci and yeasts. El-Mardi (1988) agreed with him. Abdelgadir *et al*. (2001) investigated the microorganisms associated with the fermentation of rob and reported that the microbial group isolated from this fermentation included the lactic acid bacteria *Lactobacillus fermentum, Lactobacillus acidophilus*, *Lactococcus lactis,* and *Streptococcus salivarius* and the yeasts *Saccharomyces cerevisiae* and *Candida kefyr.*

According to Naersong and Kitamoto (1995) the investigation of Edosensuu (a traditional fermented milk in Inner Mongolia in China) showed that the predominant lactic acid bacteria were *Lactococcus* and *Leuconostoc* species whereas the isolated yeast species were *Saccharomyces*. Beukes *et al*. (2001) examined the microbiology of traditional fermented milk samples collected from individual households in rural areas of South Africa and Namibia. They showed that lactic acid bacteria were the predominant bacteria in the microflora. In a similar study Savadogo *et al*. (2004) found that the

main microorganisms involved in Fulani traditional fermented milk in Burkina Faso were genus *Lactobacillus* (32%) following by *Leuconostoc* (30%), *Lactococcus* (20%), *Leuconostoc/ β-bacterium* (10%), *Streptococcus* (6%), and *Enterococcus* (2%) .

In a comparison study on microbial conditions between traditional dairy products and that produced by modern dairies, AL-Tahiri (2005) reported that the traditional dairy products showed high viable count of coliform, yeast and mold, and *Staphylococcus aureus* while that produced by modern dairies showed a very high quality of microbial standard.

Many researchers reported about the type of microorganisms isolated from the traditional fermented milk. They agree that the microflora were mainly lactic acid bacteria (Hamama and Bayi 1991; Isono *et al*., 1994; Watabe *et al*., 1998; Yoneya *et al*., 1999; Nakamura *et al*., 1999; Mathara *et al*., 2004 and Abdalla, 2007).

2.4.2 Microbial counts of fermented milk

Microbiological analysis of fresh dadih (Indonesian traditional fermented milk) by Hosono *et al*. (1989) showed that the bacterial counts ranged from 3.8 to 4.3×10^{8}cfu/g with lactic acid bacteria predominating (4.0×10^{8} cfu/g) and the major lactic acid bacteria was

Leuconostoc paramesenteroides, whereas the total yeast count was 1.1×10^{7} cfu/g. Hosono *et al*. (1990) found that the dominant and minor species of lactic acid bacteria isolated from Bulgarian ropy cultured milk were identified as *Leuconostoc dextranicum* and *L. paramesenteroides,* respectively. In further investigation of fresh dadih, Zakaria *et al*. (1998) found the bacterial count of fresh dadih was 2.3×10^{9} cfu/ml while the isolates were classified into four genera and identified as *Enterococcus, Lactococcus, Leuconostoc* and *Lactobacillus.* The investigation of *airag* (Mongolian traditional fermented milk) showed that lactic acid bacteria and yeast counts were 10^{5}-10^{7} and 10^{4} -10^{6} cfu/ml, respectively (Naersong *et al*., 1996).

Samolada *et al*. (1998) studied the changes in microbial flora during the manufacture of traditional fermented milk from ewe's milk (sour milk in eastern Greece). In the final product they found the mean log counts of lactic acid bacteria, lactobacilli and streptococci were 4.55, 3.19 and 5.56, respectively, and increased significantly to 8.46, 6.98 and 8.46, respectively at 5 days of storage and at the same time the pH decreased to 4.71. Levels of total counts, coliforms, and psychrotrophs increased to 10^{8}, 10^{7} and 10^{6} cfu/ml respectively, while halotolerant bacteria and yeasts were counted at quite low numbers

throughout manufacture. Nakamura *et al*. (1999) examined two samples of maziwa lala (a traditional fermented milk product of the Masai community in Kenya) produced by spontaneous fermentation for 2 and 7 days and noted the bacterial counts were 5.2×10^{7} and 1.3×10^{9} cfu/ml, respectively. The predominant lactic acid bacteria isolated were *Lactococcus lactis subsp. lactis, Leuconostoc mesenteroides subsp. mesenteroides, Leuconostoc dextranicum, Lactobacillus curvatus, L. paramesenteroides* and *L. plantarum*.

The investigation of Laban Zeer (fermented milk prepared traditionally in the farmhouse in southern regions of Egypt) by Ibrahim *et al*. (1999) showed that the total counts of aerobic bacteria averaged 2.4×10^{8} cfu/ml; average count of yeast and mold was 3.8×10^{7}cfu/ml. *Bacillus* was the predominant microbial group (40% of isolates), followed by *Lactobacillus, Streptococcus* and *Actinomyces* (16.7%, 13.3% and 13.3%, respectively).

Ashenafi (1995) found that at the end of culturing ergo (a traditional Ethiopian fermented milk) the average counts of coliform bacteria, lactic acid bacteria and yeasts increased to 10^{6}, 10^{9} and >10 cfu/ml, respectively. The microbial flora was dominated by

lactobacilli while the average pH and lactic acid content were 4.3 and 0.88%, respectively.

2.4.3. Fermentation of camel milk

Camel milk for human consumption is usually drunk immediately after milking and also consumed as fermented milk made by natural lactic souring over several hours in a skin or clay container. Butter is sometimes separated by vigorous shaking of the traditional fermented milk; the acid skimmed milk is drunk and the butter used for cooking, cosmetic or medicinal purposes (Yagil, 1982).

Camel's milk is often described as not easily fermented and that its butter cannot be easily extracted. Some researchers reported that camel's milk contains twice as much bacterial inhibitor (lysozyme) as that of cow's milk. At any rate many communities in Africa and Asia fermented camel's milk (Hartly, 1980; Knoess, 1980; Mukasa, 1981; FAO, 1982). Farah *et al*. (1990) studied the preparation and consumer acceptability tests of fermented camel milk in Kenya and reported that the *Susa* (traditional fermented camel milk) can be improved by using selected mesophilic lactic acid bacteria.

In an unpublished data Dirar (1970s) showed that repeated microscopic examination of Gariss (traditional fermented camel milk

in Sudan) samples obtained from the Butana and Hawawir, always revealed the presence of rod-shaped, non-sporing bacterial cells as single cells, pairs or short chains. He stated that the organisms responsible for the production of the acid and the ethanol in gariss were more likely to be lactic acid bacteria and yeasts. In 1993, the same author reported that the pH of gariss ranged from 3.4 to 3.7 and the ethanol content was about 2.0%. The microbial isolates included *Lactobacillus helveticus* and the yeast *Candida*. In an important study, Mirghani (1994) investigated the microorganisms associated with the fermentation of gariss in Sudan, and reported that the microbial isolates from this product included the lactic acid bacteria *Lactobacillus helveticus* and *Lactobacillus delbrueckii sub. lactis* and the yeasts *Candida* and *Kluveromyces.* In a recent study, Abdel-Moneim *et al*. (2006) investigated the microorganisms associated with garris (Sudanese traditional fermented camels milk product) and found that the major genera were *Lactobacillus* (74%), followed by *Lactococcus* (12%), *Enterococcus* (10%) and *Leuconostocs* (4%).

Lore *et al*. (2005) investigated the microflora involved in production of suusac, a Kenyan traditional fermented camel milk

product. They found that the lactic acid bacteria counts (LAB) were 6.8 $\log_{10}$ cfu/ml, while yeast and mould counts were relatively lower (2.1 $\log_{10}$ cfu/ml). Low coliform numbers were encountered (<1 $\log_{10}$ cfu/ml). The LAB species were identified as *Lactobacillus curvatus*, *Lactobacillus plantarum*, *Lactobacillus salivarius*, *Lactococcus raffinolactis* and *Leuconostoc mesenteroides* subsp. *mesenteroides*. The isolated yeasts were identified as *Candida krusei*, *Geotrichum penicillatum* and *Rhodotorula mucilaginosa.* The most frequently isolated species was found to be *L. mesenteroides* subsp. *mesenteroides* (24% of total isolates), followed by *C. krusei* (20%) and *L. plantarum* (16%).

A comparative study on the fermentability of camel and cow milk by lactic acid cultures (yogurt culture, cheese culture and *lactobacillus acidophilus*) was carried out by Gran *et al*. (1991). The results indicated that the 3 cultures were less active in camel milk than in cow milk, as determined by the amount and rate of acid production in the 2 milk samples. *Lactobacillus acidophilus* was found to be the least active of the 3 cultures. Colonies of the culture organisms taken from camel milk were found to be smaller than those from cow milk, possibly indicating the presence of growth- inhibiting factors in camel

milk. Camel milk failed to reach a gel-like structure (typical of cow milk) after 18 h incubation.

Attia *et al*. (2001) investigated the ability of dromedary skim milk to form an acid curd during fermentation by starter culture. They found that the dromedary milk coagulum (pH 4.4) did not reveal curd formation but indicated a fragile and heterogeneous structure, the coagulum seems to be made up of dispersed casein flakes.

The quality of acidophilus milk made from cow milk was superior and had firm curd while that made from camel milk was watery and precipitated in the form of flocks with no curd formation and the acceptability of the acidophilus milk made from cow milk scored higher than that of camel milk (Abu-Tarboush, 1994).

Rao *et al*. (1970) reported that cheese could be successfully produced from camel milk, only after mixing with the milk of goat, ewe or buffalo.

Mehaia (1993a) reported that fresh soft white cheese with good acceptability can be made from low fat camel milk with lactic cultures. However, the yield of cheese was very low compared to the cheese made from cow or buffalo. In a subsequent study, the same author (1993b) showed that an acceptable Domiati cheese with a

satisfactory gross composition yield, good flavor and overall acceptability can be obtained in cheese made from a mixture of camel milk and cow milk as follows: 50%camel milk and 50% cow milk, or 25% camel milk and 75% cow milk.

Raw camel milk showed poor rennetability and the curd formed was looser and weaker than curd from cow and goat milk (Bayoumi, 1990; Hafez and Hamzawi, 1991). Some authors have reported that camel milk cannot be coagulated with rennet unless it is mixed with other milks (Rao *et al*., 1970). Others noted that camel milk can be coagulated by itself, but only by using high dosage of calf rennet (Gast *et al*., 1969; Chapman, 1985).

Camel milk whey may be used to make acidified drinks; these drinks have an excellent nutritive value because of the presence of essential amino acids, lactose, lactic acid, vitamins and minerals. Microorganisms can use the amount of lactose in the whey as raw materials for production of some organic acids (lactic acid) during fermentation. The production of lactic acid by fermentation of camel and cow's wheys using lactic acid bacteria was studied by Qassem and Abu-Tarboush. (2000). The results indicated that at the end of fermentation (28h) the sweet camel whey, sweet cow whey and acid

camel whey had similar concentrations of lactic acid (14.22, 13.58, and 14.08 g /l, respectively), while the pH of whey samples decreased with increasing fermentation time until 12h then it remained constant.

The fermented milk contains lactic bacteria that reinforce antimicrobial activities against pathogenic agents such as *Bacillus, Pseudomonas, Mycobacterium, Staphylococcus, Salmonella and Escherichia* (Puzyrevskaya, *et al*., 2000).

2.4.4. Traditional fermented camel milk products

Fermented camel milk products have various names in various parts of the world; in the Caucasus it is called Kefir, in Armenia, Matzoon; in India, Dahdi; in Sardinia, Gioddu; in Bulgaria, Yogurt; in Sudan Gariss; and in Syria, Palestine and Egypt Laban. The traditional method for preparing fermented camel milk consists of heating milk to the boiling point so as to kill bacteria then cooling it to body temperature, and a small amount of previously fermented milk is added as a starter. The milk is well stirred and kept overnight at ambient temperature, and by the next morning it would be curdled (Aggarwala and Sharma, 1961; Kambe, 1986).

2.4.4.1. Gariss

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. In the absence of a starter from a previous lot, particularly when using a new si'in, fermentation is initiated by adding few seeds of black cumin and one onion bulb to the container, and once the first batch of gariss is successfully obtained, following the addition of fresh camel milk to the bag, gariss can be continuously produced for months (Dirar, 1993).

2.4.4.2. Suusac

Suusac is fermented camel milk widely consumed by the pastoralist communities living in the arid and semi-arid regions of Kenya and Somalia. The product is a white, low-viscosity product with a distinct smoky flavour and a stringent taste. Fresh camel milk was collected into a pre-smoked gourd and left to ferment naturally at ambient temperature (26-29°C) for 1-2 days. The top cream layer was skimmed off and the Suusac stirred (Lore, *et al*., 2005).

2.4.4.3. Chal (*Shubat*)

Chal or shubat is a white beverage that has a sour flavour, the technology of making chal is very simple; the fresh camel milk is poured in a skin bag or a ceramic jar, normally with a capacity of 30 kg. Previously soured milk is added to the fresh camel milk and mixed well. The addition of fresh milk to the mixture is continued for 3-4 days till the end product has a volume of 3-5 times that of the original chal. Shubat has a snow-white colour, thicker and fatter than kumis, its fat content reaches 8% and it can be preserved for long times without losing its properties. Shubat is used to cure tuberculosis and some gastric and intestinal diseases (Yagil, 1982).

2.4.4.4. Kefir

Kefir is a traditional fermented camel milk produced by the fermentative activity of kefir grains (which mainly consist of *Saccharomyces kefir, Torula kefir*, *Lactobacillus caucasicus, Leuconostoc spp,* and lactic acid bacteria in a protein-polysaccharide matrix). The bacteria control acid production while the yeasts produce alcohol, and the final concentration of lactic acid and alcohol may be as high as 1.0%. Camel milk is pasteurized at 85ºC then cooled to 26-

30°C and inoculated with a 3-6% of kefir culture and bottled. After incubation at 20-26°C for 8 to 12 hours, the product is allowed to ripen for 24-28 hours at 6 to 8°C; the final product is white, without gas and has a refreshing flavour and a thick creamy consistency (Vedamuthu, 1982).

2.4.4.5. Koumiss

Koumiss is similar to kefir except that mare's or camel milk is used for its production, and the culture organisms are not from kefir grain activity but the fermentation is carried out by *L. bulgaricus* and *torula* yeast. Alcohol content of koumiss varies from 1 to 2.5% while the lactic acid varies from 0.7 to 1.8%. The physical appearance of the product is unusual because mare's milk has a low level of casein thereby giving a very weak acid curd. In Russia koumiss has a clearly therapeutic significance and is widely used for treating pulmonary tuberculosis and is apparently effective. More than 50 Russian sanatoria offer koumiss treatment (Mann, 1989. Kosikowski, 1982).

2.4.4.6. Oggtt

Oggtt is a dried fermented camel milk product made and marketed primitively under uncontrolled conditions in the Arabian Peninsula. It is made by cooking or salting cow or camel milk. The

cooked type is produced by allowing the milk to ferment naturally for 1-2 days then churning and boiling the residual butter milk while stirring until it thickens. The thick paste is cooled and shaped manually into small cakes or balls which are pressed and tied in a cloth and left to dry in direct sunlight for several days. The salted type is prepared as above and then adding about 10% salt instead of heating, this type of oggtt is common in the northern region of the Arabian Peninsula, Syria, Jordan and Lebanon (Al-Ruqaie *et al*., 1987).

2.4.4.7. Acidophilus milk

Acidophilus milk is traditional milk fermented with *Lactobacillus acidophilus*, and has been considered to have therapeutic benefits in the gastrointestinal tract. It is often produced from skim or whole cow milk and from camel milk sometimes. The milk is heated to a high temperature, e.g., 95ºC for 1 hour, to reduce the microbial load and favour the slow growing of *Lactobacillus acidophilus* culture. Milk is inoculated at a level of 2-5% and incubated at 37ºC until it is coagulated. Some acidophilus milk has acidity as high as 1% lactic acid, but for therapeutic purposes 0.6-0.7% is more common. Nutrients such as honey, glucose and tomato

juice may be added to stimulate bacterial growth. It has the merit of being the best natural fermented milk because the microorganism responsible for fermentation easily colonizes and can have beneficial effects on the human intestine (Veena *et al*., 1986).

2.5. Biochemical changes in fermented milk

2.5.1. Chemical composition of fermented milk

Many workers around the world investigated the chemical composition of fermented milk. Hamama and Bayi (1991) showed that the average composition of forty two Raib (Moroccan traditional fermented milk) and Jben (Moroccan traditional fresh cheese) was 10.7 and 37.5% total solids, 2.22 and 16.47% fat, 3.1 and 15.8% protein, 4.2 and 4.1% lactose, 0.17 and 0.5% chloride and 0.54 and 1.265 ash, respectively, while the pH and lactic acid content were 4.2 and 4.1, 0.67 and 1.04%, respectively. Chemical composition of leben (Iraqi fermented milk) was 3% fat, pH 3.9, 1.31% titratable acidity, 0.07% ethanol and 339 mg acetoin diacetyl/kg (Abo-Elnaga *et al*., 1977).

Musaiger *et al*. (1997) analyzed fermented dairy products commonly consumed in Bahrain and reported that the chemical

composition depended on the type of milk, method of preparation, type and proportion of starters and consumer preferences.

In Sudan the proximate analysis of robe sample collected from Khartoum market was shown to contain 7.2% total solids, 3.3% protein, 2.0% lactose, 0.16% fat, 1.9% total acidity (as lactic acid) and a pH value of 3.5 (Saeed, 1981; El–Mardi, 1988). In a subsequent study, Dirar (1997) reported that the pH and the acidity of freshly prepared rob were 4.9 and 0.76%, respectively.

The chemical composition of Laban (the popular fermented milk in Middle East countries that comes under different names that is made from the milk of cows, sheep, goats or camels) was extensively analyzed by many workers. In Oman (Guizani *et al*., 2001) reported that Omani home-made Laban had an average titratable acidity, pH, fat, protein and total solids of 1.12%, 3.98, 1.12%, 2.11% and 6.29%, while the commercial Laban had 0.77%, 4.52, 3.5%, 3.45% and 10.47%, respectively. In Iraq (Moussa *et al*., 1984) showed the average chemical composition of Laban on dry matter basis as follows: 16.46% T.S, 31.95% fat, 34.78% protein, 38.36% lactose, 4.8% ash with 1.89% acidity and a pH 3.65.

According to many investigators the chemical composition of *Madeer* or *Oggtt* (a traditional cultured camels milk in the Arabian peninsula) is 3.9% water, 35.5% protein (N×6.38), 15.3% fat, 7.9% ash and 0.5% fiber (Sawaya, 1984b; Al-Mashhadi *et al*., 1987; Al-Mohizea, *et al*., 1988).

Mirghani (1994) found that the chemical composition of *Gariss* (a traditional fermented camel milk in Sudan) is as follows: 1.35-1.4% lactose, 2.15-2.9% fat, 3.4-3.85% protein, 0.75-0.8% ash, 91.7-92.65% moisture, 1.3-1.4% ethanol, 0.13-0.2% volatile fatty acids, 1.0-0.8% total acidity (as lactic acid) and a pH value of 3.25-3.40.

2.5.2. Proteolytic activity

All starter culture species are nutritionally very fastidious requiring many amino acids and growth factors for adequate growth. The lactic acid bacteria are only mildly proteolytic compared to, for example, *Bacillus* and *Pseudomonas*, which have a complex proteolytic system capable of converting milk casein to the free amino acids and peptides necessary for growth and acid production. The essential amino acids for growth of lactic acid bacteria in milk are either absent or present at concentrations too low to support their growth in milk (Law and Haandrikman, 1997). A study by Mills and

Thomas (1978) revealed that the free amino acids and low molecular weight peptides present in milk can support the growth of *Lactobacillus lactis subsp. cremoris* to cell densities corresponding to 8-16% of those found in coagulated milk. The composition of amino acids in milk products fermented with thermophilic lactic *streptococci* or acidophilic rods was studied by Muradyan *et al*. (1976).The results showed that these fermenting microorganisms enriched the resulting products with at least 4 amino acids (cysteine, valine, proline and arginine).

The strains of lactic acid bacteria used in the preparation of fermented milks are characterized by a specific proteolytic activity that assumes high biological significance because it can substantially modify casein solubility in yoghurt. During yoghurt production amino acids are also released so that the amount that exists free in the yoghurt is obviously higher than the amount in the original milk (Groux, 1972). In another study Rasic *et al*. (1971) found that a significant increase is reached in the biological value of the proteins during the preparation of yoghurt. In a study conducted at the University of Agricultural Sciences, Bangalore, India, the amounts of free amino acids in *Dahi* ranged from 0.2 to 38.0 mg/100g. Highest

concentrations of free amino acids were found in *Dahi* prepared with mixed cultures containing *Streptococcus cremoris* and *Streptococcus thermophilus*. (Laxminarayana, 1976).

Many authors studied the proteolytic activities of the lactic acid bacteria. Sasaki *et al*. (1995) tested the proteolytic activities in various lactobacilli from raw milk and various milk products. They found that *Lactobacillus* strains have a higher proteolytic activity than the *Lactococcus* strains. In a similar study 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 the most proteolytic of all organisms used. In a similar study Shihata and Shah (2000) screened the proteolytic activity of nine strains of *S. thermophilus*, six strains of *L. bulgaricus*, 14 strains of *L. acidophilus* and 13 strains of *Bifidobacterium*, he found that the amount of free amino acids group released by *S.thermophilus, L. bulgaricus* and *L. acidophilus* were higher than that by *Bifidobacterium* strains.

Proteolytic activities of nine strains of *Streptococcus thermophilus* and nine strains of *Lactobacillus bulgaricus* cultures incubated in pasteurized reconstituted milk at 42ºC showed that lactobacilli were more proteolytic and produce more acid than

streptococci whereas the mixed cultures except one combination liberate more tyrosine (Rajagopal and Sandine, 1990). In a similar study Ibrahim (1998) tested the proteolytic activity of seven mesophilic starter cultures grown separately in sterilized skim milk for 8h at 25ºC. He observed that the short-chain-forming cultures had high proteolytic activities, whereas the long-chain-forming cultures had low activities.

The autolysis and proteolysis in different strains of starter bacteria during chedder cheese ripening were studied by Wilkinson *et al*. (1994). The results stated that different *Lactococcus* had different autolysis patterns during ripening, the effects of which on ripening and flavor have not yet been clearly demonstrated. In a recent study Carrasco *et al*. (2005) noted that *Lactobacillus* strains were highly proteolytic (60-169µg tyrosine ml^{-1} of milk) and *Streptococcus* strains were less proteolytic (18-31µg tyrosine ml^{-1} of milk) while strains belonging to the *Lactobacillus* genus showed a great acidifying capacity.

Abu-Tarboush (1994) observed that the proteolytic activities of *Lactobacillus acidophilus* in camel milk were similar to its activities in cow milk. In a subsequent study in 1996, the same author found

that the proteolytic activities of the yogurt cultures strains (four strains of *Streptococcus thermophilus* and three strains of *Lactobacillus delbrueckii ssp. bulgaricus)* were higher in camel milk than in cow milk. Whereas mixed cultures released the same amount of free amino groups as the corresponding single cultures, except for *L. delbrueckii ssp. bulgaricus LB12.*

The composition of free amino acids of camel milk and *Shubat* (3 or 10 day cultured camel milk) was investigated by Sulaimanova *et al*. (1998). The results indicated that there was a 3- fold increase in free amino acids, particularly tyrosine, valine, cysteine, phenylalanine, aspartic, glutamic acids, proline and histidine in *Shubat* compared to camel milk while tryptophan and asparagine were present in *Shubat* only. He underlined the increase in the amount of amino acids in shubat could be due to the effect of lactic acid bacteria.

The proteolysis activity of four species of bifidobacteria except *B.longum 15707* was higher in fermented camel milk than bovine milk (Abu-Taraboush *et al*, 1998).

2.5.3. Effect of fermentation on milk fat

Milk fat is the most variable of all milk components, and is recognized as major contributer in determining consumer acceptability

of most dairy products (Day, 1960). Triglycerides form 95-99% of fat in milk, and 60 to 70% of the fatty acids are unsaturated, 25 to 35% monounsaturated, and about 4% polyunsaturated. The remaining 1 to 2% of milk fat is composed of phospholipids, sterols, carotenoids, fat-soluble vitamins and some traces of free fatty acids (Kurtz, 1974).

The investigation of lipase activity from lactic acid bacteria used as pure cultures in the dairy industry is important because these enzymes may make important contributions to the flavor of fermented dairy foods. According to various authors the lactic acid bacteria have a hydrolytic action on triglycerides with a short chain but the action is still slower than that which could be exerted on triglycerides with longer chains. The lipolytic activity that may be present in yoghurt can be almost exclusively attributed to lipolytic enzymes possessed by the culture starters, for example during storage of yoghurt the limited hydrolysis of fat occurs regularly and has been proved by Formisano. (1974) who established that the free fatty acids in yoghurt are dominated by those with a long chain and have a profile similar to those in milk. Fermentation of whole milk by *Lactobacillus acidophilus, L. bulgaricus,* and *Streptococcus thermophilus* resulted in a significant increase in the level of saturated fatty acids and oleic acid

with a decrease in linoleic and linolenic acids. The increase in the level of free fatty acids was small; the changes in cholesterol levels were also not significant but there were significant increases in the levels of stearic and oleic acids. Results indicate that previously reported hypocholestermic effect of fermented milk is not due to changes in the composition of investigated lipid classes (Rao and Reddy, 1984). Similarly Alm (1982a) found that differences between unfermented and fermented milk products are small for the relative composition of fat (mono-di-and triglycerides, free fatty acids and steroids) and for the fatty acid profile following hydrolysis. In another study Oberman (1985) found that the lipase activity of lactic acid bacteria influenced the changes in fatty acid pattern and fatty acid pool. Singh *et al*, (1973) reported that the lipases from *L. casei* and *L. lactis* were found to be intracellular.

Enzymes with lipolytic activities have been identified in a number of lactic acid bacteria and their commercial application in dairy foods had been well studied by Adams and Brawley (1981) and Hill (1988).

Meyers *et al*. (1996) tested over 100 different lactic acid bacteria for lipase production and reported that lactic acid bacteria

were found to produce lipases, but they were weakly lipolytic when compared with other microorganisms such as *Pseudomonas, Aeromonas, Acinetobacter* and *Candida.*

2.5.4. Effect of fermentation on lactose

Lactose is the major carbohydrate of milk in most mammals and its concentration represents about 40, 50 and 70% of the solids in whole milk, skim milk and whey, respectively (Nickerson *et al*., 1975). The content of lactose in camel milk ranges from 3.4 to 5.6%, which is slightly higher than its contents in cows milk (Farah, 1993).

Alm (1982b) observed that after 11 days storage the lactose content of yogurt decreased to about 2.3% compared to 4.8% in nonfermented milk, while galactose content increased from traces in milk to 1.3% in yogurt. Similar results were found with acidophilus and bifidus milk.

Saitmuratova and Sulaimanova (2000) analyzed samples of camel's milk and its fermented product (*Shubat*). They reported that the carbohydrate content of camel's milk was 16-16.5%, while that of shubat was 3-5 times lower. Both milk and shubat contained glucose, galactose and insignificant amounts of mannose.

Toba *et al*. (1983) investigated yogurt prepared by fermentation with

L. bulgaricus and *St. thermophilus* and found that lactose content decreased from 6.53 to 4.22%, galactose content increased from 0.04 to 1.46%while glucose and oligosaccharides increased from 0 to 0.04% and 0.08%, respectively.

Brien (1999) studied the sugar profile of cultured dairy products in the UK and confirmed that the most lactic acid fermentations result in a decrease in lactose content and an increase in free galactose.

2.5.5. Effect of fermentation on vitamins

During fermentation the lactic acid bacteria require vitamins for growth particularly during the rapid augmentation phase and they are able to synthesize vitamins again during the following phase .For example, yogurt cultures of *Streptococcus thermophilus* and *Lactobacillus bulgaricus* result in about 90% decrease in B12 content. There are occurring smaller losses of other B vitamins, for vitamin B2 and biotin a partial synthesis is supposed and the vitamin C content is scarcely changed (Renner, 1983). In another study, Reif *et al*. (1976) clearly showed the capability of lactic acid bacteria, used in the production of cottage cheese, to carry out vitamin synthesis. Niacin and vitamin B6 increase slowly with time while vitamin B12 and folic acid are synthesized rapidly. In general fermented milk products

showed an increase in folic acid content and slight decrease in vitamin B12 while other vitamins B were affected slightly (Alm, 1982c).

Czarnocka and Wojewodzka (1969) reported that vitamin C in vitamin-fortified milk had been shown not to be utilized during fermentation with yogurt microflora. The low pH value of the product had a favorable influence on the stability of vitamin C, so that losses during storage were very small. However, Bonczar and Regula (2003) found that the vitamin C content decreased in ewe's milk after pasteurization and in yogurts during storage period.

The effect of heat processing on milk vitamins was discussed by Lavigne, *et al*. (1989). They found those losses in thiamine riboflavin and total vitamin C in High Temperature Short Time (HTST), flash, and Ultra-High Temperature (UHT) processes were less than those in Low Temperature Long Time (LTLT), and autoclave processes. Mehaia (1994) found reduction in vitamin C of about 27, 41,53, and 67% for samples of raw camel milk heated for 30 min at 63, 80, 90, and 100ºC, respectively.

Bambha *et al*. (1973) reported that inoculation of milk with *Propionibacterium shermanii* along with lactic acid cultures resulted in an increase in vitamin B12, riboflavin, thiamine and folic acid

contents of dahi (Indian fermented milk). Further, at the stage of *Dahi* preparation there was an increase in the content of vitamin B2 (121-131%), folic acid (165-331%) and niacin (160-210%). In contrast, during *Chakka* preparation, there were substantial losses of all 3 vitamins in whey. The product made with *St.thermophilus* and *L. bulgaricus* was superior in nutrient content to that made from *Streptococcus lactis-40* (Atreja and Deodhar, 1987).

Shahani and Chandan (1979) reported that cultured milk products contained higher levels of folic acid, niacin, biotin, pantothenic acid, vitamin B6 and vitamin B12 than fresh milk. However in another study the biological activity of lactic acid bacteria resulted in a decrease of about 50% in vitamin B6, B12 and vitamin C level, while only small changes in vitamin A, B1, B2 and niacin took place (Oberman, 1985). In contrast, Hartman and Dryden (1965) think that several cultures are capable of synthesizing certain vitamins: for example, some strains of lactic bacteria have been found to augment the vitamin B12 content of the product by 20-30% or more. That means the changes in vitamins content are dependent on the kind of microorganisms, the time and temperature of incubation.

In *Amaas* (soured milk in Southern Africa), only the amount of nicotinic acid was reduced, with no changes in the contents of other vitamins (Golberg *et al*., 1945).

Platt (1964) stated that fermented milks are good sources of the B vitamins, including vitamin B12, the original amount of which may be increased by the process of fermentation.

The combination of starter microflora, bifidobacteria, *Lactobacillus bulgaricus* and kefir starter at a ratio of 1: 0.5: 0.5 in fermented milk products increased the content of thiamine and riboflavin by 27 and 18%, respectively, while all the strains individually increased folic acid by 71-100% (Khamagaeva et at., 1986). On the other hand, Baranova *et al*. (1998) reported that fermentation of goat milk by selected lactic acid bacteria did not influence tocopherol contents, but there was a slight decrease in thiamine and riboflavin content, and a significant decrease in vitamin C, except where *L. casei* was used the vitamin C content increased by 25%. Similarly, Saidi and Warthesen (1993) observed that yogurt fermentation carried out in the laboratory for 5 h reduced the riboflavin content.

2.5.6. Organic acids and ethanol

Organic acids occur in dairy products as a result of hydrolysis of buttermilk by bacterial growth. Determinations of these acids in dairy products is important to flavor studies, for nutritional reasons, and as an indicator of bacterial activity, furthermore ,organic acids are proven natural preservatives; lactic acid has been correlated to the inhibition of certain pathogenic bacteria in yogurt (Rubin *et al*, 1982).

Homofermentative lactic acid bacteria ferment carbohydrate predominantly to lactic acid with formation of only trace amounts of ethanol and carbon dioxide whereas some homofermentative lactobacilli, however, produce considerable amounts of acetic acid (Marth, 1962; Dirar and Collins, 1972; Rasic and Kurman, 1978).

Determination of volatile acids and ethanol in Swedish dairy products showed that acetic acid was low in yogurt but high in bifidus milk, other products showed intermediate acetic acid. Ethanol content was low in all fermented products except for kefir, which initially contained about 20 mg/100g, and following storage increased to 200mg/100g (Alm, 1981). In Egypt, Damir *et al*. (1992) detected six organic acids during *kishk* fermentation (fermented food made by

mixing wheat with fermented milk) and lactic acid had the highest increment rate while formic acid had the lowest.

In sterilized skim-milk fermented with lactic acid bacteria, a total of 7 organic acids, including the original organic acids present in skim-milk were detected; levels of citric, lactic and acetic acids varied during storage; the citric acid content decreased after 24h fermentation whereas the lactic and acetic acid content increased (Kato *et al*., 1992).

3. Materials and Methods

3.1. Sources and maintenance of cultures

Culture strains used in this study were obtained as lyophilized pure cultures from Chr.Hansen's Laboratorium. (Hørsholm, Denmark A/S) *Streptococcus thermophilus 37*, *Lactobacillus delbrueckii sp.bulgricus CH2, Lactococcus lactis, Lactobacillus acidophilus* and mixed yogurt culture (*S.thermophilus and L. bulgaricus* 1**:**1) were used. 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 the Gram stain.

3.2. **Preparation of fermented milk**

Fresh whole camel milk from *Camelus dromedarius* was obtained from a private herd. Milk was immediately cooled and kept at 5±1^{o}C during transportation to the laboratory for one hour. The whole camel milk was pasteurized in 500-ml quantities at 80^{o}C for 15min in a water bath and cooled immediately to 5±1^{o}C in an iced bath. The milk samples (500ml) were equilibrated for one hour at the fermentation temperature (43ºC) in a water pat before inoculation with the starte 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 18-24h 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 *Streptococcus thermophilus 37, Lactobacillus delbrueckii ssp. bulgaricus CH2, Lactococcus lactis, Lactobacillus 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 hours of incubation were analysed for chemical and microbiological quality. The whole experimental procedure was repeated three times using three different batches of milk.

3.3. Methods

3.3.1 Microbiological Analyses

3.3.1.1 Preparation of samples

Fermented camel milk samples (11ml) were homogenized for one minute in 99ml ($^1/_{10}$) of a sterile solution of 0.I% (w/v) peptone water (Oxoid CM9) using a Stomacher Lab blender (Model

400,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).

3.3.1.2. Enumeration of aerobic mesophilic bacteria

Viable aerobic mesophilic bacteria were enumerated in Plate Count Agar (Oxoid CM325) following the pour – plate method and incubated in an inverted position at 32^{o}C±1 for 48 hour in an electrical incubator (Memmert Gm – Germany). After 48h plates were counted using colony counter (Model 3327-American Optical, USA) then results were reported as standard plate count (SPC) per ml sample (Houghtby *et al*., 1992)

3.3.1.3. Psychrotrophs count

Psychrotrophic bacteria were enumerated in Plate Count Agar (Oxoid CM325) and plates were incubated at 7^{o}C for 10 days. The plates were then counted using a colony counter (Model 3327-American Optical, USA) and the results were reported as standard plate count (SPC) per ml sample (APHA, 1992).

3.3.1.4. Total coliforms count

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^{o}C ±2 for 18-24h. The plates were counted and reported as Coliform Plate Count (CPC) per ml sample (Mehlman, 1984).

3.3.1.5. Enumeration of lactic acid bacteria

The lactic acid bacteria were enumerated in pour plates of de Man, Rogosa and Sharp (MRS) medium (Oxoid CM359) according to Gilliland *et al*. (1984).

The plates were incubated at 37^{o}C for 48h under microaerobic conditions using Gas Pak (H_2+CO_2) anaerobic systems (BBL, Microbiology Systems, Div. Becton Dickinson and Co., Cockeysville. Med.). After incubation, the plates were counted and reported as Standard Plate Count (SPC) per ml sample.

3.3.1.6. Enumeration of Yeasts and molds

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). The acidification of the medium is necessary to provide for suppression of bacterial growth by adjusting the medium to pH 3.5±0.1.Duplicate plates were used for each count. Pour plates were inverted and incubated at 25^{o}C±1 for 3-7 days and results were reported as SPC per ml fermented milk (Koburger and Marth, 1984).

3.3.1.7. Enumeration of Staphylococci

The total Staphylococci were enumerated by plating onto Baird-Parker Agar (OxoidCM275) following the pour plate method. The medium was cooled to 46^{o}C and supplemented with an egg yolk-tellurite emulsion (Oxoid CM54) 50ml per liter medium. The incubation temperature used was 37˚C±1 for 36h. At the end of the incubation period the plates were counted and the result was reported as total Staphylococci per ml sample (Marshall, 1992).

3.3.1.8. Enumeration of Proteolytic bacteria

The proteolytic bacteria were estimated using the plate count agar (PCA, Oxoid CM325) pour plate technique. Decimal dilutions of the sample were inoculated in duplicate plates of PCA medium, which contained 10% of sterile skim milk. The inverted plates were incubated for 72h at 30°C±1°C then the plates were flooded with 10% acetic acid or 1% HCl. After 60 seconds the excess acid was removed,

and colonies with white or off-white zones of precipitation round them were considered to be proteolytic bacteria and were enumerated. Flooding of the plates with acid is necessary as some acid-forming bacteria are also capable of producing clear zone (Frank *et al*; 1992).

3.3.1.9. Detection of pathogens in fermented products

The detection of *Salmonella spp., Staphylococcus aureus, Bacillus cereus, Listeria monocytogenes* and *E. coliO157: H7* were applied according to the methods described in the FDA (1998).

For *Salmonella spp.* detection 25 ml of fermented milk was pre-enriched in 225ml of buffered peptone water (Oxoid CM509) at 37ºC for 24h, 0.1 ml of the pre-enrichment sample was then incubated in 10 ml of Rappaport –Vassiliadis medium (Oxoid 669) at 43ºC for another 24h. Enrichment was then streaked onto Xylose Lysine Deoxycholate (XLD medium-Oxoid) plates and incubated at 37ºCfor 24h. Presumptive positive colonies of *Salmonella* were tested using the rapid API 32E, system (Biomerieux, France).

Staph. aureus was detected by plating on Baird-Parker Agar (Oxoid-CM 275) following the surface plate method. Inoculated plates were incubated at 37°C for 24-48 h. Dark grey, shiny convex colonies with a white margin surrounded by a clear zone, were identified as

presumptive *S. aureus*. Suspected colonies were confirmed by API 32 Staph system (Biomerieux, France).

The detection of *Listeria spp*. was performed by the following procedure: a 25 ml sample was homogenized with 225ml of Listeria selective enrichment broth (Oxoid CM897 with SR141 Supplement) and incubated at 37ºC for 48h. The enrichment was then streaked on Listeria selective agar (Oxoid CM856 with SR 140 supplement) and the plates were incubated at 37ºC for 24-48h. Colonies which showed a morphology typical of *Listetria spp.* were tested by the API Listeria system (Biomerieux, France). Modified *E. coli* broth with novobiocin ($_{m}$E C+ n) was used as the basal enrichment medium and sorbitol MacConkey agar was used as the selective plating medium for enumeration identification of *E. coli O157: H7*.

The *Bacillus cereus* count was determined by the surface plating method with Mannitol Egg Yolk Polymyxin (MYP) Agar (Lancette and Harmon, 1980), and the plates were incubated at 30ºC for 24 h for enumeration. Typical colonies of *Bacillus cereus* were then transferred to nutrient agar slants and identification was confirmed by microscopic and biochemical characterization that includes Gram stain and API 50 CH B system (Biomerieux, France).

3.3.2. Chemical analysis

3.3.2.1. Fat content

The Fat content of the milk was measured according to Gerber's method. From an automatic measure, 10ml of sulphuric acid with density 1.815 to 1.820 g/ml was taken into the empty bottle, commonly called a "butyrometer". Eleven ml of properly mixed milk sample was slowly transferred into the bottle by holding the pipette in a slant position and allowing the milk flow to touch inside the wall of the butyrometer, so that it forms a layer on the top the sulphuric acid. Next 1ml of amyl alcohol of density 0.803 to 0.810 g/ml at 20^{o}C was added into the bottle by using an automatic tilting measure. Then the lock stopper was fixed and the contents of the butyrometer were vigorously shaken until the curd completely dissolved. The bottle was kept in a water bath at 65±2^{o}C for 5 minutes and centrifuged for 5min at 1000 to 1100 r.p.m. after balancing the centrifuge. The fat column in the butyrometer was adjusted suitably and the fat percentage in the milk was directly read and noted (Atherton and Newlander 1982).

3.3.2.2. Determination of Total protein

Total nitrogen was determined by a modification of the AOAC procedure (1995). Triplicate 5 ml aliquots of samples were pipetted

into separate 300 ml Kjeldahl flasks. To each flask, 2g of mixed catalyst (10 parts K_2SO_4 and 1 part $CuSo_4$), 2 boiling chips and 10 ml of concentrated sulphuric acid were added. Samples were digested until clear with the addition of antifoam B as needed to retard foaming. The samples were cooled, distilled, titrated and total nitrogen was calculated according to standard AOAC procedure (1995) Total protein was calculated by multiplying total nitrogen by a factor of 6.38.

3.3.2.3. Determination of Lactose

The lactose content was determined according to the method described by Abu-Lehia (1987). Five ml of trichloroacetic acid (TCA) was added to five ml of fermented milk sample and mixed thoroughly. The sample was then filtered through Whatman No. 40 filter paper and two ml of the clear filtrate was diluted to 100ml with distilled water. One ml of diluted filtrate was transferred to a 15ml tube fitted with a screw cap. Two and a half ml of diluted working solution (two days shelf life working solution was prepared by mixing one volume of 1% phenol, two volumes of 5%sodium hydroxide, two volumes 1% picric acid and one volume of fresh 1% sodium disulfide solution then the working solution diluted 1:1 with distilled water according to

Teles *et al*. (1978), was added to the tube contents and mixed thoroughly. The tube was stoppered and immersed to a depth of 4 to 6 cm in a vigorously boiling water bath exactly 2.5 min. Then the tube was cooled under cold tap water and 7ml of distilled water was added to the tube contents and mixed thoroughly. The absorbance at 520 nm was measured against a blank containing all reagents except that milk was replaced by distilled water. Volumes of fat and protein in the samples were corrected as described by Grimbleby (1956). A standard curve from different concentrations of anhydrous lactose in distilled water was prepared for this method by plotting absorbances against the corresponding lactose concentrations.

3.3.2.4. Determination of moisture content and Ash

The moisture content was estimated by drying 5g of fermented milk at 105˚C to a constant weight, while the ash content was found by heating 5 grams of milk in a muffle furnace at 550˚C for 24h.

3.3.2.5. Measurement of pH and titratable acidity

To determine the pH of fermented milk samples, a pH meter (Orion Research Inc., Cambridge, MA, USA.) was first standardized using pH 7 and 4 buffers. The samples were mixed gently and the electrode was dipped into the samples, when the value displayed was

steady, the pH value was recorded. Between samples, the electrode was rinsed with distilled water and wiped with tissue.

The titratable acidity was determined by titrating 10 ml of fermented milk against 0.1 N NaOH to the phenolphthalein end point expressed as percent lactic acid.

3.3.2.6. Measurement of proteolytic activities

The proteolytic activities of the cultures were determined spectrophotometrically, by additions of 10 ml of 0.75N TCA and 1ml of water to 5ml 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. The standard curve was prepared using Leu-Gly (Sigma Chemical Co., St Louis, MO) at 20 to 200µm concentrations. The OPA reagent was prepared essentially as described by Goodno *et al*. (1981). The OPA solution was made by combining the following reagents and diluting to a final volume of 50ml with water: 25ml of 100mM sodium tetraborate; 2.5ml of 20% (wt/wt) sodium dodecyl sulfate (SDS); 40mg of OPA

(dissolved in 1 ml of methanol); and 100µl of ß-mercaptoethanol. This reagent was prepared daily. To assay proteolysis with milk protein as substrates, a small aliquot (usually 10 to 50µl containing 5 to 100µg protein) was added directly to 1.0 ml of OPA reagent in a 1.5 ml quartz cuvette; the solution was mixed briefly by inversion and incubated for 2 min at ambient temperature, and the absorbance at 340 nm was measured in a spectrophotometer.

3.3.2.7. Fatty acids analysis

Milk fat was extracted according to the Rose-Gottlieb method (AOAC, 1984). Triplicate extractions were carried out for each sample. The extraction solvents were evaporated at 60-70^{o}C until the milk fat was obtained. The milk fat was then stored in a refrigerator until analysis.

All chemical solvents used in the extraction were analytical grade [Ammonia solution, Ethyl alcohol 95%, Ethyl ether and Petroleum ether (40-60^{o}C)] .

Fatty acid methyl esters (FAMEs) were prepared following the procedure described by Metcalfe *et al*. (1966). Aliquots of lipid extract (20mg) were saponified with 1.5ml methanolic KOH (0.5N) solution by refluxing for 10min at 85˚C. After addition of 4ml boron

triflouride methanol complex reagent (20% BF_3 in methanol), the sample was boiled for another 5 min. The FAMEs were thrice extracted from salt saturated mixture with petroleum ether (40-60^{o}C). Thin-layer chromatography showed that complete methylation was achieved. The esters were separated by gas chromatography (GC) (HP5890 A.USA) fitted with a capillary column. Supelcowax 10. 30m×0.32 id. 0.5oμm film thickness (Supelco. Belletone, PA. USA). The oven temperature was programmed from 110-185^{o}C at 2˚C/min. and then increased to 220^{o}C at a rate of 4C/min with final hold time 5 min. Injector port and flame ionization detector temperature were 250 and 260^{o}C., respectively. Helium was used as a carrier gas at inlet pressure 1.2 kg/cm^2. Six standard mixtures of 20 pure FAMEs (Supelco and Sigma) and cod liver oil esters were injected to confirm the identification. Standards were routinely chromatographed to establish retention times in order to determine the response factor for the individual fatty acids. All FAMEs were run in duplicate. Pentadecanoates were used as an internal standard. Fatty acid profile was quantitated according to procedures outlined in AOCS (1977).

3.3.2.8. Determination of amino acids

Fermented samples in duplicate were hydrolyzed with 6N HCl for 24 hours at 110^{o}C (Moore and Stein, 1963). For Amino acids analysis all the hydrolysates were performed on reverse phase–high-pressure liquid chromatography (Shimadzu 34 LC – 10 AD, Shimadzu corporation, Kyoto, Japan) following hydrolysis of one gram each sample with 20 ml 6N HCl for 24h at 110^{o}C in capped tubes under nitrogen (AOAC, 1995). Individual amino acids were separated on Shimpack amino – Na type column (10cm x6.0mm) obtained from Shimadzu Corporation. The samples were post column derivatized with O-phthaldialdehyde (OPA) and detected with fluorescent detector RF- 10A Shimadzu at Ex. 350 nm and Em450 nm. Data were recorded and integrated using an integrator model C-R7A (Shimadzu chromatopac data processor).

3.3.2.9. Determination of sugars

Samples were prepared as described by Pirisino (1983). Five ml of fermented milk, five ml of water, and 20 ml of HPLC grade acetonitrile were added to a 50 ml round-bottom glass centrifuge tube, shaken 1 min, and centrifuged for 10 minutes at 5000 r.p.m. [Universal Centrifuge Model PLC-012, Germany Industrial Corp.] To

obtain a clear supernate. An aliquot of the supernate was clarified by passing it through a 0.45-µm-membrane filter. Ten to 15 µl were injected. Triplicate analysis was performed for all samples.

The clear supernatant was analyzed using HPLC Shimadzu LC. NH_2 from Shimadzu, Kyoto-Japan.The mobile phase (20% water and 80% acetonitrile, HPLC grade) was introduced by a delivery pump model LC-10 AD (Shimadzu) at a flow rate of 2.5 ml/min. The system was attached to an injector (Model SIL –10A, Shimadzu) through which a 5µL sample was injected. The running time was 15 min. The peak areas for the calibration curves and for the calculations of sugar amounts in the samples were measured by an integrator model C-R7A (Shimadzu chromatopac data processor). Sugar standards were purchased from Sigma (Sigma Chemical Co., St. Louis, Mo.). Results were reported as a percentage (w/w).

3.3.2.10. Determination of organic acids

For determination of organic acids and ethanol, 10g of fermented milk were centrifuged at 10000 r.p.m. for 20 min. The supernatant was filtered through membrane filter 0.45µm diameter 25mm (Schleichen ∝ Schiill-Germany) and analyzed by HPLC Shimadzu LC. NH_2 from Shimadzu, Kuoto-Japan using an organic

acid column PL Hi-plex H (from Polymer Laboratories Amherst, M.A. 01002, U.S.A) fast acid column, mobile phase 0.001M H_2SO_4 at 57°C flow rate 0.7ml/minutes. Results were reported as percentage (w/w) (Marsili *et al*., 1981).

3.3.2.11. Determination of Vitamin C (Ascorbic acid)

Vitamin C (Ascorbic acid) was determined by 2,6-dichloroindo phenol titrimetric method as described in AOAC (1984).

Ascorbic acid reduces oxidation - reduction indicator dye 2,6-dichloroindophenol to colorless solution. At the end point excess unreduced dye is rose pink in acid solution. Vitamin is extracted and the titration is performed in the presence of metaphosphoric acid-acetic acid, to maintain acidity.

Reagents

a- Extraction solution: Metaphosphoric acid- acetic acid solution (15g of metaphosphoric acid was dissolved in 40ml acetic acid and 200ml of distilled water, then diluted to 500ml and filtered.

b- Ascorbic acid standard solution (mg/ml).

c- Indophenol standard solution 50mg 2,6 dichlorophenol was dissolved in 50ml H_2O, contained 42mg sodium bicarbonate then diluted to 200ml with H_2O and filtered.

Procedure

The dye was standardized by taking 1ml of standard ascorbic acid to which was added 6ml of the acid, then titrated to the end point. A blank titration of the acid was performed by taking 7ml of the acid then, titrated with the dye. Duplicate samples of 2ml volume of milk to which 5ml of metaphosphoric – acetic acid was added then titrated with the dye to the end point.

3.3.2.12. Determination of Riboflavin (Vitamin B_2)

Riboflavin (vitamin B_2) was determined by fluorometric method as described in AOAC (1984).

Reagents: Riboflavin stock solution 100 µg/ml, working standard, 0.05 µg/ml, 0.1µg/ml.

Five ml of milk samples in duplicates were diluted to 10ml with distilled water then acidified to PH (5-6) with 0.1 N HCl, then 1ml of 10 N HCl was added to each, refluxed by heating at 120^{o}C for 30 min for complete hydrolysis. The hydrolyzates containing riboflavin were diluted to 10 ml with distilled water and 2.5 ml of concentrated acetic acid were added to each, then centrifuged at 4000 rpm for 10 minutes. The fluorescence of the supernatant was measured at 440 mm

(excitation filter) and 565 mm (emission) {Perkin Elmer MPF -44B. Spectrofluorometer, Input filter 440mm, Output filter 565mm}.

The concentrations of the standard and the samples were printed out directly and the concentrations of the samples were calculated according to the standard curve.

3.3.2.13. Determination of Thiamine (Vitamin B_1)

Thiamine (Vitamin B_1) was determined by fluorometric method as described in AOAC (1984).

Reagents: 2.5mg thiamine HCl (previous by dried at 105^{o}C, cooled in a disiccator) was transferred into 100ml volumetric flask then dissolved in 30ml 20% aqueous alcohol and the pH was adjusted to 4 with dilute HCl. The concentration of the stock was 25 µg/ml.

Working standard concentration of thiamine (0.2 µg/ml) was prepared by diluting 0.8 ml stock thiamine to 100ml with dilute HCl (1/60).

Oxidizing reagent: 4ml of 1% potassium ferricyanide was completed to 100ml with 15% sodium hydroxide.

. Dry isobutyl alcohol.

Procedure:

Five ml of standard solution (0.2 µg/ml thiamine) and 5 ml of sample supernatant were transferred into clean dry test tube, to each tube 3ml of the oxidizing agent (containing 4ml of 1% potassium ferricyanide completed to 100ml with 15% sodium hydroxide) was added with mixing ,then extracted with 20ml isobutyl alcohol by vortexing for 1.5 minute. Ten ml of the alcohol layer (top) was removed from each tube and the fluorescence was determined by using the fluorometer at 365mm input (excitation) and 435mm output (transmittance).

A blank sample was prepared from isobutyl alcohol and determined.

Calculation: Standard concentration = Standard reading – Blank

Sample reading = Sample assay reading – Blank reading

D: Dilution factor

$$\text{Sample concentration} = \frac{\text{Standard concentration x Samples reading x D}}{\text{Standard reading}}$$

3.4. Sensory evaluation

Samples of fermented camel milk by different strains of lactic acid bacteria were evaluated by 10 consumer panel (all of them were familiar with fermented camel milk- Gariss) using a 9-point hedonic rating scale (9 = excellent; 7 = good; 5 = acceptable; 3 = poor;

1 = extremely poor). Sensory evaluation took into consideration the attributes of color, smell, taste, consistency and overall acceptability, also the panelists were asked to list any defects. The fermented samples for sensory evaluation were prepared as follow: Five hundred ml of pasteurized camel milk was fermented by adding 5% (V/V) of starter cultures then incubated at 43^{o}C for 6 hours then cooled immediately to 7 - 10 ^{o}C in an iced bath.

The fermented samples were served at temperatures 7-10^{o}C using white 50ml plastic cups labeled with three-digit codes from a random number table. Panelists received a tray containing samples, a glass of water to rinse their mouths between samples, crackers to aid in removing beany flavour between tasting and an evaluation form with pencil at the beginning of the evaluation.

3.5. 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).

4. Results and Discussion

4.1. Raw Camel milk

4.1.1. Proximate analysis of the camel milk

The proximate analysis of the raw camel milk used in this study is shown in Table (2).

The moisture content was 88.3%, fat 3.30%, protein 3.00%, lactose content 4.62% and the ash content was 0.77 %.

These results are similar to those found by El-Amin and Wilcox (1992) who reported the following proximate analysis of the camel milk in Saudi Arabia 88.33% moisture, 3.15% fat,2.81% protein, 4.16 lactose and 0.83% ash content, and with Sawaya *et al*. (1984a) who gave the following proximate composition of camel milk: moisture 88.3%, protein 3.00%, fat 3.80%, lactose 4.7% and ash 0.8%. Values of this research work are also within the range reported by Dirar (1993) except for the protein content (3.3-4.7%) which is higher than that obtained in this study (3.00%).

Results are comparable with those reported in various parts of t che world by many researchers, Mirghani (1994) in Sudan, Abu-Lehia (1987) and Mehaia *et al*. (1995) in Saudi Arabia, Gnan and Sheriha (1986) in Libya and Sankhla *et al*. (2000) in India).

Table (2): Chemical composition of camel milk

Sample	Moisture %	Fat %	Protein %	Lactose %	Ash %	pH	Total Acidity %
Camel milk	88.3± 0.100	3.30± 0.100	3.00± 0.15	4.62± 0.34	0.77± 0.02	6.64± 0.09	0.15± 0.01

● Results are means of triplicate determinations ± S.
*Expressed as percent lactic acid

4.1.2. Total titratable acidity and pH

The total acidity (expressed as percent lactic acid) and pH of the raw camel milk in the present study are shown in Table (2). The total acidity was 0.15% and the pH was 6.64. These results are similar to those reported by Mirighani (1994) and Sawaya *et al*. (1984a) who found pH 6.5, 6.5 and total acidity 0.17, 0.13 respectively. In general comparable results also were reported by Abdel-Rahim (1987); Auru (1987); Dirar (1993) and Mehaia *et al.* (1995).

4.1.3. Vitamin C, Riboflavin and Thiamine Content

The mean values of the vitamin C, riboflavin and thiamine contents of the raw and pasteurized (80ºC for 15 min) dromedary camel milk, used in this study, are shown in Table (3).

The level of vitamin C (22.54 mg/kg) was close to that reported by Sawaya *et al*. (1984a), Knoess (1980) and Mehaia (1994) whose reported 23.7, 23.8 and 24.9 mg/kg respectively, but was quite lower than those reported by Sohail (1983) and Farah *et al*. (1992) who gave the values of 58.2 and 37.4 mg/kg respectively.

As shown in Table (3), the riboflavin content was 0.41 mg/kg and thiamine content was 0.31mg/kg. These results are similar to those found by Sawaya *et al*. (1984a) who reported 0.42 for riboflavin and

Table (3): Vitamin C, thiamine and riboflavin content (mg/kg) of raw and pasteurized camel milk

Sample	Vitamin C	Riboflavin (B2)	Thiamine (B1)
Raw camel milk	22.54±8.50	0.410±2.45	0.31±0.80
Pasteurized camel milk (80ºC for 15 minutes)	15.32±2.20	0.392±1.60	0.296±1.50
% loss	32.03%	4.39%	4.51%

● Results are means of triplicate determinations ± S.D

0.33 mg/kg for thiamine of camel milk, but substantially lower than those given by Knoess (1980) who gave values for riboflavin and thiamine contents for camel milk 0.80 and 0.6 mg/kg, respectively. In another study Farah *et al*. (1992) reported 0.6mg/kg for riboflavin content in fresh camel milk which is also higher than our result. Pasteurization of camel milk at 80ºC for 15 min. resulted in losses of vitamin C, riboflavin and thiamine of about 32.03%, 4.39% and 4.51%, respectively. These findings were in agreement with that reported by Mehaia (1994) who found decreases in vitamin C of about 27, 41, 53, and 67% for samples of raw camel milk heated at 63, 80, 90, and 100ºC for 30 min, respectively, with a negligible amount of destruction (4-7%) of the riboflavin content. Similarly Lavigne, *et al*. (1989) found losses in thiamine, riboflavin and total vitamin C as a result of heat processing of milk.

4.1.4. Amino acid composition

Amino acid composition of the dromedary camel milk is shown in Table (4).

Table (4): Amino acid composition of casein in camel milk (g/100g)

Amino acid (g/100g protein)	Content in camel milk
Aspartic acid	6.89±0.16
Threonine	4.21±0.24
Serine	4.28±0.21
Glutamic acid	18.86±0.21
Proline	11.26±0.04
Glycine	1.32±0.10
Alanine	2.27±0.08
Valine	6.93±0.28
Methionine	3.03±0.04
Isoleucine	5.14±0.05
Leucine	8.42±0.00
Tyrosine	4.39±0.11
Phenylalaline	4.65±0.21
Lysine	6.68±0.23
Histidine	2.31±0.30
Arginine	3.47±0.18

- Results are means of triplicate determinations ± S.D

In general, the amino acid composition of camel milk used in this study was in agreement with those reported by Mehaia and Al-Kanhal (1989), also the result round in this research work is comparable to the results found by Sawaya *et al*. (1984a) and Farah and Ruegg (1989), except for glutamic acid (23.9, 21.26), Aspatric acid (7.6, 7.28), Leucine (10.4, 10.89), serine (5.8, 5.39) and threonine (5.2, 4.87), respectively which are higher than those found in this study.

4.1.5. Fatty acid composition

The data presented in Table (5) show the fatty acid composition of camel milk used in this study.

Results showed that palmitic (26.5%) and oleic (24.2%) acids were the major fatty acids present followed by stearic (12.2%), myristic (10.2%) and palmitoleic (9.98%) acids. The findings in this research are similar to those reported by Abu-Lehia (1987) and comparable to those found by Sawaya *et al*. (1984a) and Gorban and Izzeldin (2001), but it was lower than that reported by Yagil (1982). Data revealed that the short chain fatty acids (C4–C12) were present in very small amount while the concentrations of C14:0, C16:0 and

Table (5): Fatty acid composition of camel milk fat

Fatty acids	Molar % of total fatty acids
Butyric acid C4	0.63±0.021
Caproic acid C6	0.36±0.028
Caprylic acid C8	0.19±0.014
Capric acid C10	0.13±0.007
Lauric acid C12	0.91±0.014
Myristic acid C14	10.2±0.141
Peutadecanoic acid C15	1.55±0.014
Palmitic acid C16	26.5±0.353
Palmitoleic acid C16:1	9.98±0.296
Stearic acid C18	12.2±0.282
Oleic acid C18:1	24.20±0.141
Linoleic acid C18:2	3.21±0.134
Linolenic acid C18:3	1.35±0.056
Arachidic acid C20	2.10±0.141

● Results are means of triplicate determinations ± S.D

C18:0 are relatively high. This finding was in agreement to the general pattern of the camel milk fatty acids reported by Hagrass *et al*. (1987), Abu-Lehia (1989), Farah *et al*. (1989), Farag and Kebary (1992), Mohamed and Hjort (1993).

4.1.6. Microbiological analysis of raw milk

Table (6) shows the total aerobic bacterial count, psychrotrophs, total staphylococci count, total proteolytic bacteria, total coliform count and total yeasts and molds count of the raw camel milk used in this study expressed as cfu/ml.

The mean counts expressed as cfu/ml were $1.4x10^5$ total aerobic count, 4.2×10^3 total staphylococci, 1.3×10^2 psychrotrophs, 6.9×10^2 total proteolytic bacteria, 2.7×10^2 total coliform and <10 total yeasts and molds in the raw camel milk.

Results showed that total aerobic count (1.4×10^5 cfu/ml) was within the range of those reported by Adam (1987), (9.2×10^2 to 4.2×10^7 cfu/ml), Farid (1987), (8.3×10^3 to 2.4×10^4) and Mohizea (1986) (1.7×10^2), while total coliform and total staphylococci were within the range of the results found also by Al-Mohizea (1986) for total coliform (6.0x10-7.3×10^4 cfu/ml), staphylococci (1- 8.4×10^3

Table (6): Microbiological analysis of raw camel milk

Microbial group	Count Cfu / ml
Total mesophilic aerobic bacterial	$1.4\times10^{5}\pm0.021$
Total Staphylococci	$4.2\times10^{3}\pm0.021$
Total Proteolytic bacteria	$6.9\times10^{2}\pm0.084$
Total Coliform	$2.7\times10^{2}\pm0.028$
Total Psychrotrophs	$6.9\times10^{2}\pm0.084$
Total Yeasts and Molds	$<10\pm0.0$

● Results are means of triplicate determinations ± S.D

cfu/ml) in raw camel milk. The yeasts and molds counts in raw camel milk used in this study were quite lower than that obtained by Mustafa *et al*. (2000) who reported 4.5×10^2 cfu/ml in Egypt.

The previous studies of microbiological quality of raw camel milk by many researchers showed that the farmers personal hygiene and their hygiene practice in milk handling could be expected to influence the number of microorganisms in the raw milk, therefore, the bacteriological quality of raw milk may be considerably improved by strictly hygienic methods of milk production, immediate cooling of the milk and storage temperature under 4ºC (Thomasand Druce, 1971; Chatelin and Richard, 1981).

4.2. Fermented milk

4.2.1. Microbiological analysis

4.2.1.1. 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 Fig (1). The initial viable cell counts of starter cultures ranged from 4.39 (*Lactococcus 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 for the

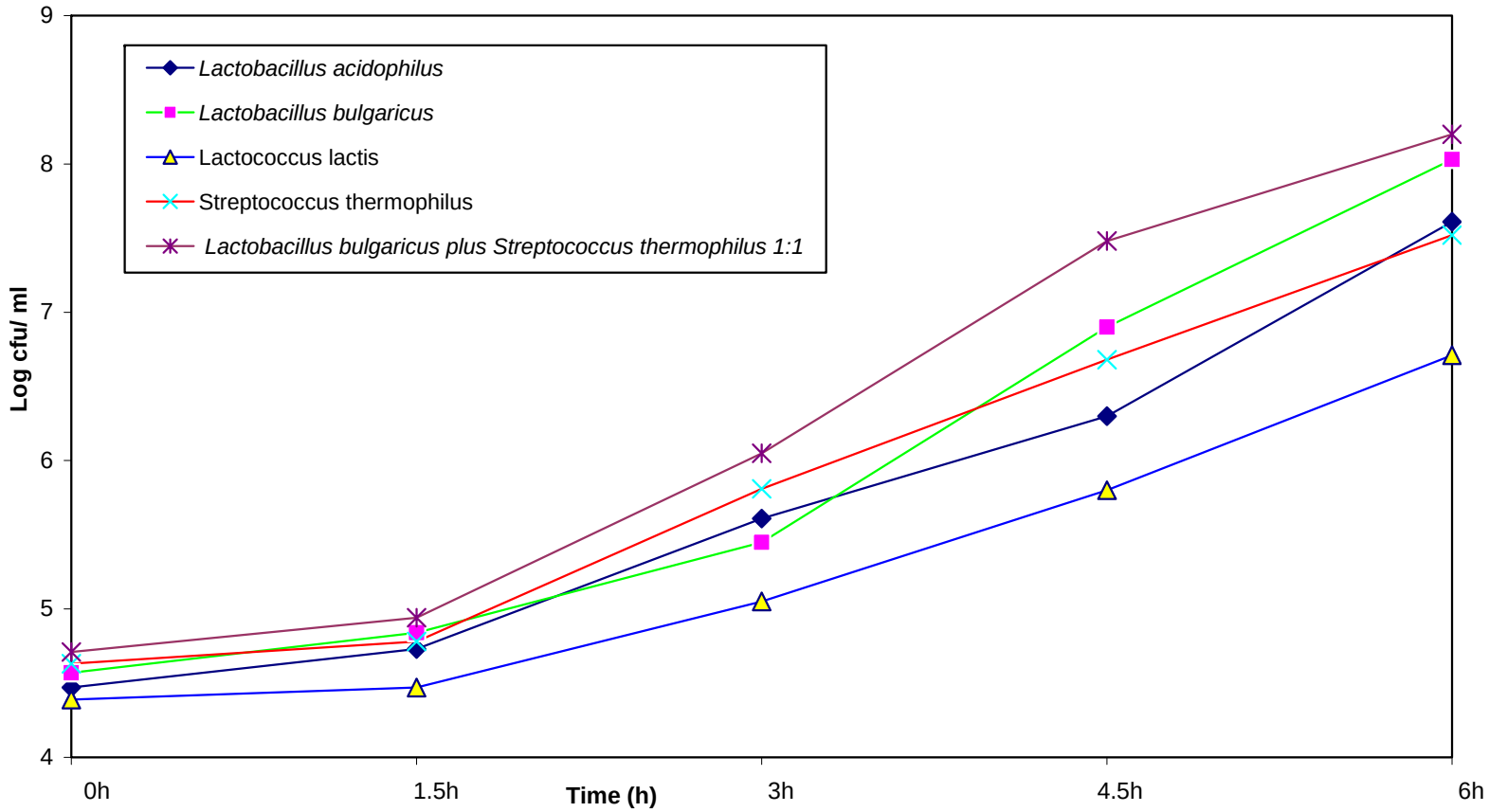

Fig (1) : The changes on the viable counts of the starter culture strains during fermentation of camel milk for 6h at 43ºC

five cultures and remained stable with minor increase after 1.5h of incubation. The average counts after 3h incubation were 5.41, 5.65, 5.05, 5.51, 6.05 and those after 4.5h were 6.3, 6,9, 5.8, 6.68, 7.48 $\log_{10}$ cfu^{-ml} for *L. acidophilus, L. bulgaricus, Lactococcus lactis*, *St. thermophilus* and combination of *L. bulgaricus* and *St. thermophilus* (1:1), respectively. At the end of the fermentation process (6h) 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 *Lactococcus 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 (6h) 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 4h.

On the other hand Abdel Moneim *et al*. (2006) have showen 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.*

4.2.1.2. Microbiological quality of fermented camel milk products

The prevalence of *Salmonella spp*., *Staphylococcus aureus*, *listeria monocytogenes, Bacillus cereus, E.coli O157:H7,* total yeasts and molds and total coliform counts are shown in Table (7).

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*., *Staphylococcus aureus*, *listeria monocytogenes, E. coli O157:H7* or *Bacillus cereus,* while the total coliform, yeast and mold counts were less than 10 cfu per ml. The absence of the pathogens was mostly 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 confirmed by Puzyrevskaya, *et al*. (2000) who reported that fermented camel milk

Table (7): Microbiological analysis of fermented camel milk products

Tests	Fermented camel milk products at 43ºC for 6h by:				
	Lactobacillus acidophilus	*Lactobacillus bulgaricus*	*Lactococcus lactis*	*Streptococcus thermophilus*	*St.thermophilus* and *L. bulgaricus* 1:1
Total coliform plate count	<10 cfu/ml	<10 cfu/ml	<10 cfu/ml	<10 cfu/ml	<10 cfu/ml
Yeast andmold plate count	<10 cfu/ml	<10 cfu/ml	<10 cfu/ml	<10 cfu/ml	<10 cfu/ml
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 25ml of sample

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 in the commercial laban samples were not detected. 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 *Staphylococcus 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, Listeria monocytogenes* and *Staphylococcus 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).

4.2.2. Biochemical analysis

4.2.2.1. 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 hours. The initial pH of the inoculated camel milk for the 5 cultures at start of fermentation was 6.25 (*L. acidophilus*), 6.22 (*L. bulgaricus*), 6.24 (*Lactococcus lactis*), 6.22 (*St. Thermo.philus*), and 6.21 (combination strains of

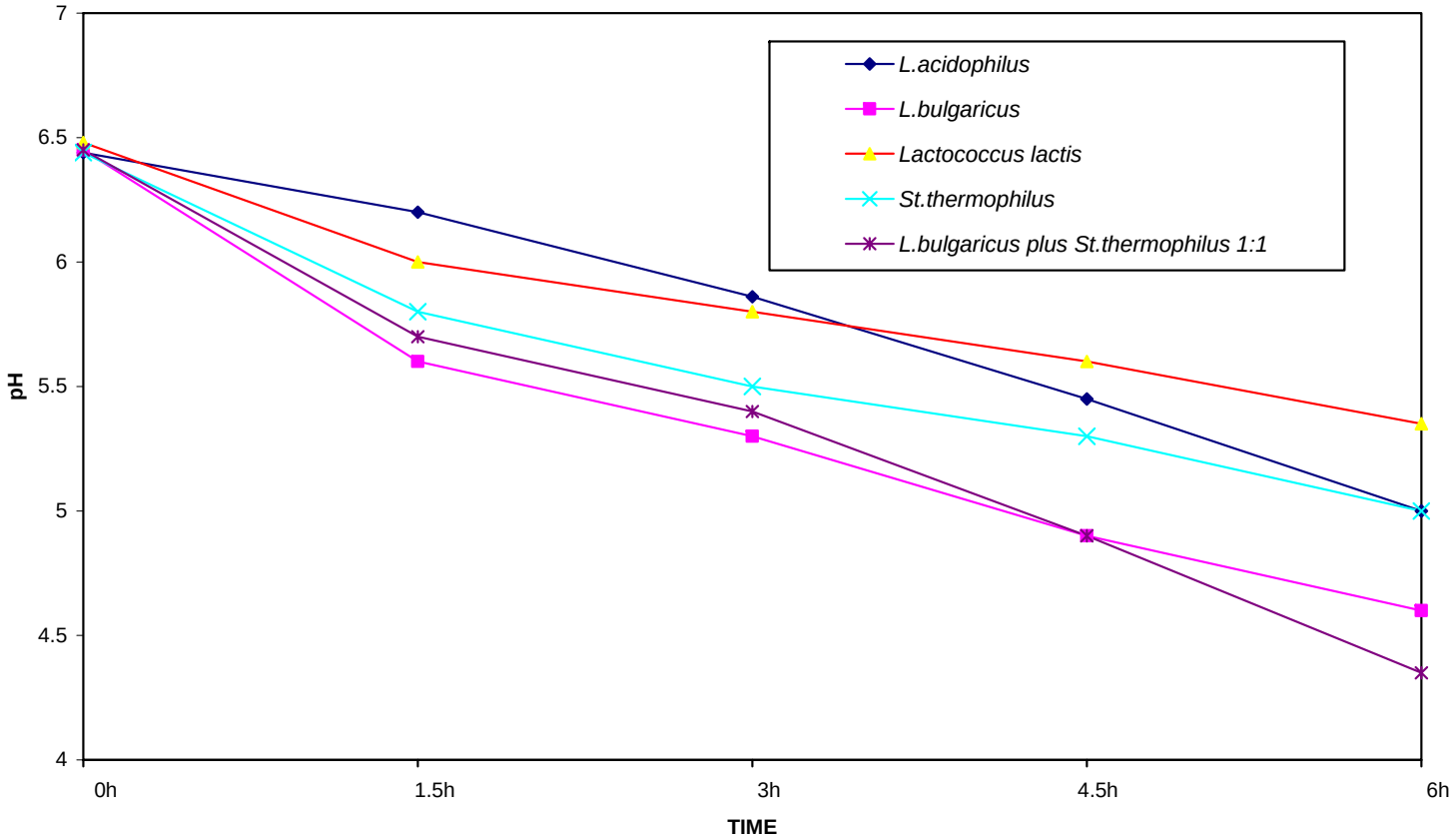

Fig (2) : pH during fermentation of camel milk by selected culture strains at 43ºC for 6h

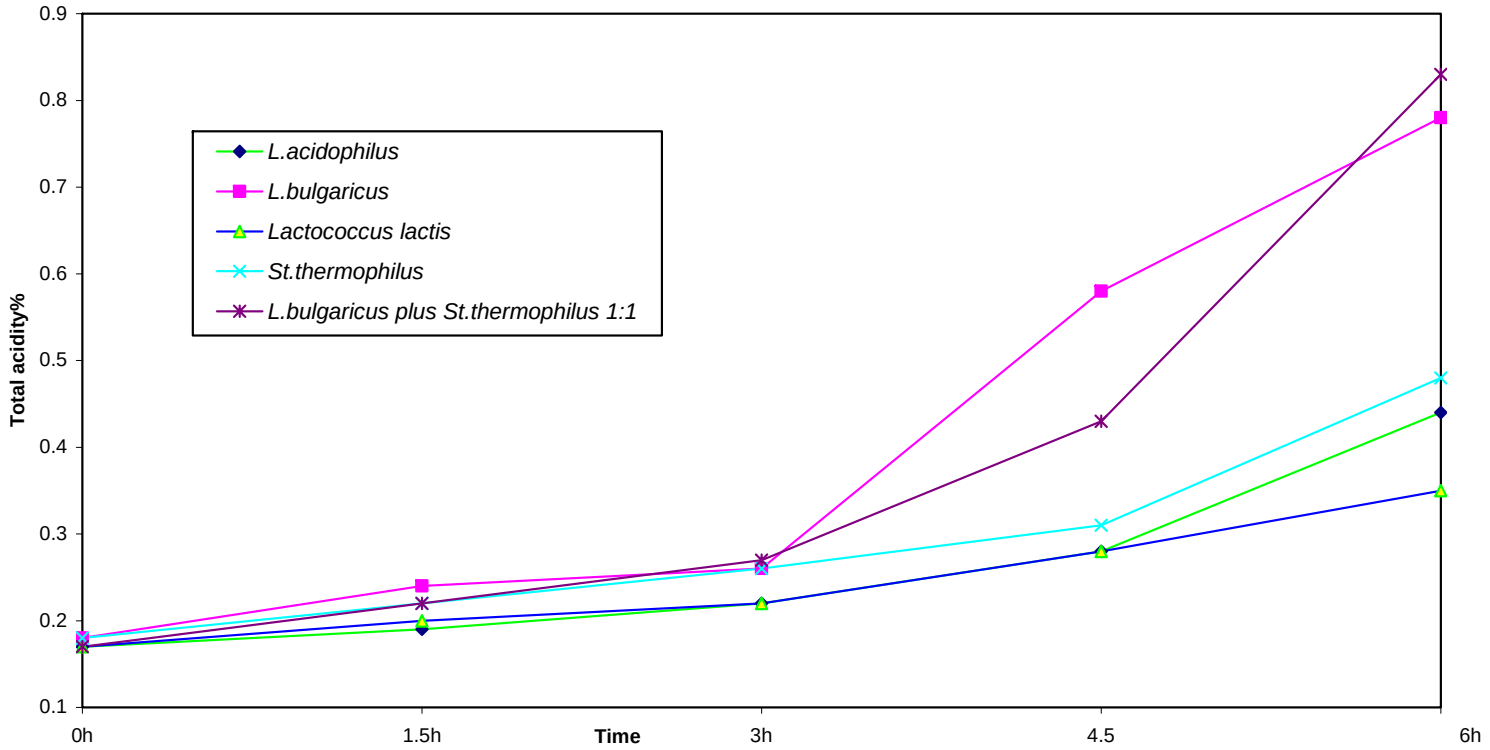

Fig (3) : Total acidity % during fermentation of camel milk by culture strains at 43ºC for 6h

L. bulgaricus and *St. thermophilus*, 1:1). After 3h of fermentation the pH decreased from 6.29 to 5.86 for *L. acidophilus,* from 6.22 to 5.50 for *L. bulgaricus*, from 6.24 to 5.80 for *Lactococcus lactis*, from 6.22 to 5.50 for *St. thermophilus* and from 6.21 to 5.40 for the combination *L. bulgaricus* and *St. thermophilus*, 1:1). At the end of fermentation (6h) the pH decreased to 5.00, 4.60, 5.35, 5.00 and 4.35 while the total acidity increased 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 previous results indicated that the pH of camel milk fermented by single culture of *L. bulgaricus* was lower than the 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 obtained by Rajagopal and Sandine (1990) and Carrasco, *et al*. (2005), who reported that the *St. thermophilus* cultures gave higher pH than the *L. bulgaricus* cultures and the pH for mixed cultures was much lower than those for the pure cultures. This finding agreed also with that observed by Abu-Tarboush (1996) who studied the behavior of

different strains of commercial cultures in whole camel milk incubated at 42ºC for 4h. He found that the final pH of *Lactobacillus 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 higher than that reported by Mirgani (1994) who gave a range of 3.9-4.0 for pH in garris (Sudanese traditional fermented camel milk). However, it was similar to that reported by Lore, et el. (2005) of suusac (pH 4.3) (Kenyan traditional fermented camel milk).

On the other hand the values of pH (5.00) and total acidity (0.44%) obtained from camel milk fermented with *L. acidophilus* at 43ºC for 6 hours were in accord with the acidophilus milk made from camel milk by Abu-Tarboush (1994) who reported 4.93 and 0.57% for pH and total acidity, respectively.

4.2.2.2 Proteolytic activities of starter cultures in camel milk

The changes in proteolytic activities of *L. acidophilus, L bulgaricus, Lactococcus. lactis*, *St. thermophilus* and mixed cultures

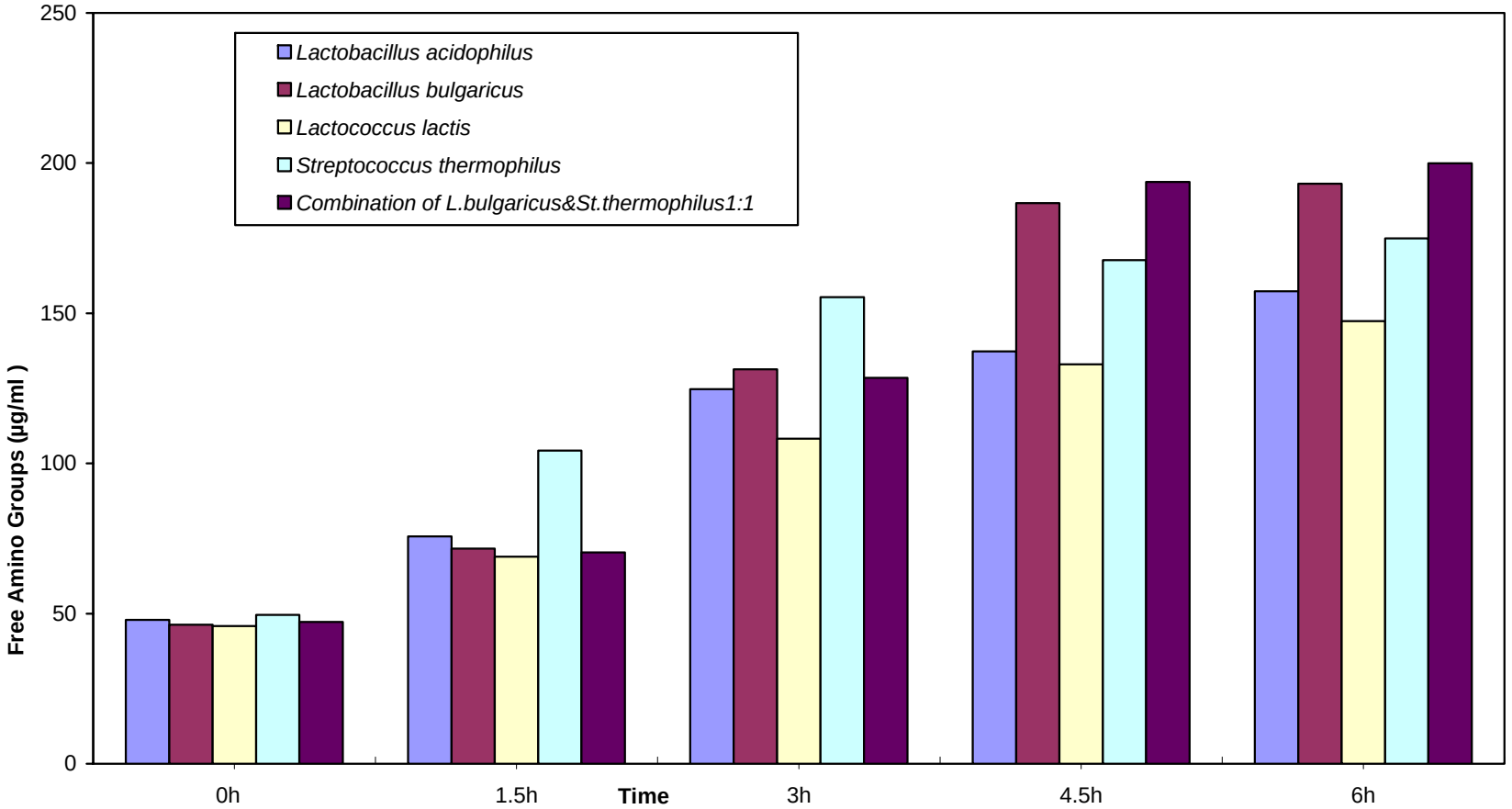

Fig (4) : Proteolytic activities of the culture strains during fermentation of camel for 6h at 43ºC.

of *L. bulgaricus* and *St. thermophilus* (1:1) during fermentation of camel milk at 43ºC for 6 hours are presented in Figure (4).

The amount of free amino groups (FAG) in the camel milk fermented for one hour and half were 75.65, 71.62, 68.92, 104.25 and 70.30 µg/ml for the five starter cultures respectively. The amounts of FAG released by these starter cultures were almost the same except for that by *S. thermophilus* (104.25 µg/ml) which was much higher compared to the other cultures. The concentration of FAG after 3h increased from 75.65 to 124.72, from 71.62 to 131.32, from 68.92 to 108.23, from 104.25 to 155.31µg/ml and from 70.30 to128.48 for the five starter cultures, respectively. These values showed that *St. thermophilus* still had the highest proteolytic activity followed by *L. bulgaricus*, mixed cultures of *L. bulgaricus* and *St. thermophilus* (1:1) and *L. acidophilus* while *Lactococcus lactis* had the lowest value.

The concentration of FAG for *L. acidophilus* were (137.28, 157.31), those for *L. bulgaricus* were (186.67, 193.14), those for *Latococcus. lactis* were (132.94, 147.37), those for *St. thermophilus* were (167.68,174.90) and those for mixed cultures of *L. bulgaricus* and *St. thermophilus* (1:1) were (193.7, 199.98 µg/ml) after fermentation of camel milk for 4 and half and 6 hours, respectively.

These results have shown that the proteolytic activity of *L. bulgaricus* was the highest one compared to *St. thermophilus*, *L. acidophilus* and *Latococcus. lactis* but it was lower than that of mixed cultures of *L. bulgaricus* and *St. thermophilus* (1:1) in the camel milk fermented at 43°C for 6 hours. The amount of FAG released by the mixed starter cultures of *L. bulgaricus* and *St. thermophilus* (1:1) in camel milk fermented at 43°C for 6 hours was greater than that released by the other single cultures (Figure 4). This observation is in accordance with that found by Sasaki *et al*. (1995) who reported that *Lactobacillus* strains have a higher proteolytic activity than the *Lactococcus* strains, 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 the most proteolytic of all organisms used. These findings also agreed with those reported by Rajagopal and Sandine (1990) who reported that the lactobacilli were highly proteolytic and streptococci were less proteolytic whereas mixed cultures always liberated more tyrosine in cow skim milk than the sum of the corresponding single cultures except for one combination, but it differed from the results reported by Abu-Tarboush (1995) who found

that the amount of FAG released by mixed cultures was almost the same as that produced by any of the corresponding single cultures.

In another study, Shihata and Shah .(2000) 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. Similarly Carrasco *et al*. (2005) concluded that the yogurt bacteria (*S. thermophilus* and *L. delbrueckii spp. Bulgaricus*) appeared to be highly proteolytic as compared to the probiotic bacteria (*L. acidophilus* and *Bifidobacterium*).

4.2.3. Chemical composition of fermented camel milk

Results obtained from the chemical analyses of camel milk fermented by five starter cultures of lactic acid bacteria for 6 hours at 43ºC are presented in Table (8).

After 6 hours incubation at (43ºC) the total solids values were 12.78, 11.64, 11.80, 11.79 and 11.88% for *L. acidophilus, L. bulgaricus, Lactococcus lactis*, *St. thermophilus* and mixed culture of *L. bulgaricus* and *St. thermophilus* (1:1) cultures respectively. These results indicated that no significant ($P>0.05$) differences were observed in total solids of the fermented milk between the five starter

Table (8): Chemical composition of camel milk fermented for 6 hours at 43ºC by selected starter cultures

Chemical composition (g /100g)	*Lactobacillus acidophilus*	*Lactobacillus bulgaricus*	*Lactococcus lactis*	*Streptococcus thermophilus*	Yogurt culture (1:1) *S.thermophilus* and *L.bulgaricus*
Moisture%	87.22 (±0.064)b	88.41(±0.113)a	88.20 (±0.064)a	88.19 (±0.198)a	88.12(±0.028)a
T.S%	12.78 (±0.141)a	11.64 ±0.212)b	11.80 (±0.282)b	11.79 (±0.141)b	11.88 (±0.141)b
Protein%	3.49(± 0.057)a	3.21 (±0.064)b	3..30 (±0.064)ab	3.39 (±0.198)ab	3.38 (±0.014)ab
Fat%	3.55 (±0.071)a	3.60(±0.00)a	3.65 (±0.071)a	3.55 (±0.071)a	3.60(±0.141)a
Lactose%	3..69 (±0.134)b	3.89(±0.042)a	3.98 (±0.127)a	4.06 (±0.078)a	3.98(±0.191)a
Ash%	0.83(±0.007)b	0.89 (±0.014)a	0.88 (±0.057)a	0.82(±0.007)b	0.82(±0.007)b

- Values are means ± SD of three replicates
- Means not sharing a common following letter in a raw are significantly different at $p<0.05$

cultures except that the value for the milk fermented by *L.acidophilus* culture was higher (12.78%).

The fat content was 3.55, 3.60, 3.65, 3.55, and 3.60% whereas the protein content was 3.49, 3.21, 3.30, 3.39, and 3.38% for *L. acidophilus, L. bulgaricus, Lactococcus lactis*, *St. thermophilus* and mixed culture of *L. bulgaricus* and *St. thermophilus* (1:1) for the five starter cultures, respectively. From the results shown in Table (8), no significant difference was observed in the fat content of fermented camel milk by the five starter cultures.

The protein content of milk fermented by *Lactococcus lactis* (3.3%), *St. thermophilus* (3.39%) and mixed yogurt cultures (3.38%) was similar while it is slightly higher for that fermented by *L. acidophilus* (3.49%) and lower for that by *L. bulgaricus* (3.21%).

The values of ash content were 0.83, 0.89, 0.88, 0.82, 0.82% and those of lactose were 3.69, 3.89, 3.98, 4.06, 3.98% for the five starter cultures respectively. The ash content of camel milk fermented by *L. acidophilus, St. thermophilus,* and mixed culture of *L. bulgaricus* and *St. thermophilus* (1:1) was comparable while, those fermented by *L. bulgaricus* and *Lactococcus lactis* were slightly higher compared to them. The lactose content of fermented camel

milk by *L. acidophilus* was slightly lower than those fermented by other starter cultures.

In general, the average values for the proximate chemical analysis of the fermented camel milk were higher in this study compared to the traditional fermented camel milk investigated by Mirghani (1994) who found that the chemical composition of gariss (a traditional fermented camel milk in Sudan) was as follows: 1.35-1.4% lactose, 2.15-2.9% fat, 3.4-3.85% protein, 0.75-0.8% ash, 91.7-92.65% moisture. These values are lower than those obtained in the present study except for protein content, which falls in the same range. These differences can be attributed to the type of fodder eaten by camels, method of preparation, type of starter cultures, time and temperature of incubation, as the gariss was based on the traditional conditions and methods while our product was prepared under laboratory conditions. Furthermore, Musaiger *et al*. (1997) concluded that the chemical composition of various types of fermented milks depended on the type of milk, method of preparation, type and proportion of starters.

On the other hand, the values obtained in the present study were comparable with those reported for a commercial fermented cow milk

called Laban in Sultanate of Oman by Guizani *et al*. (2001) who found 0.77% (T.A), 4.52 (pH), 3.5% (Fat), 3.45% (protein) and 10.47% (Total solids).

4.2.4. Fatty acid profiles of fermented camel milk

The fatty acid composition of fermented camel milk by five starters of lactic acid bacteria for 6 hours at 43ºC is given in Table (9). In previous studies enzymes with lipolytic activities have been identified in a number of lactic acid bacteria and their commercial application in dairy foods had been well studied by Adams and Brawley (1981) and Hill (1988). Meyers, *et al*. (1996) tested over 100 different lactic acid bacteria for lipase production and reported that lactic acid bacteria, were found to produce lipases, but they were weakly lipolytic when compared with other microorganisms such as *Pseudomonas, Aeromonas, Acinetobacter* and *Candida.*

As Table (9) shows, the palmatic acid was the major saturated fatty acid followed by myristic and stearic acid while oleic acid followed by linoleic acid were the major unsaturated fatty acids in the fat of the fermented camel milk by the five culture. These results indicated that no significant difference was observed between the five

Table (9): Fatty acid composition (g/100g) of camel milk fermented for 6h at 43ºC by selected starter cultures

Fatty acids (g/100g)	*Lactobacillus acidophilus*	*Lactobacillus bulgaricus*	*Lactococcus lactis*	*Streptococcus thermophilus*	Yogurt culture (1:1) *St.thermophilus* and *L. bulgaricus*
Caprylic acid C8	0.165 (±0.035)a	0.495 (±0.332)a	0.345 (±0.176)a	0.345 (±0.120)a	0.220 (±0.070)a
Capric acid C10	0.155 (± 0.035)a	0.160 (±0.000)a	0.185 (±0.007)a	0.305 (±0.191)a	0.360 (±0.240)a
Lauric acid C12	1.085 (±0.021)b	1.065 (±0.021)b	1.160 (±0.042)a	1.045 (± 0.021)b	1.090 (±0.028)b
Myristic acid C14	14.71 (±0.282)a	14.505 (±0.346)a	15.435 (±0.502)a	14.635 (±0.375)a	15.015 (±0.091)a
Peutadecanoic acid C15	1.965 (±0.261)a	2.160 (±0.070)a	1.915 (±0.502)a	2.160 (±0.057)a	2.190 (±0.084)a
Palmitic acid C16	42.79 (±0.142)a	41.935 (±0.275)a	42.960 (±0.014)a	41.945 (±0.544)a	41.975 (±0.586)a
Palmitoleic acid C16:1	1.555 (±0.049)a	1.595 (±0.091)a	1.960 (±0.367)a	1.625 (±0.007)a	1.610 (±0.014)a
Stearic acid C18	12.150 (±0.049)b	12.180 (±0.141)b	12.025 (±0.007)b	12.305 (±0.007)a	12.125 (±0.035)b
Oleic acid C18:1	29.415 (±0.120)a	29.510 (±0.989)a	28.340 (±1.541)a	28.745 (±0.883)a	29.110 (±0.212)a
Linoleic acid C18:2	3.435 (±0.176)a	3.410 (±0.070)a	3.395 (±0.190)a	3.595 (±0.262)a	3.425 (±0.134)a
Linolenic acid C18:3	1.865 (±0.007)a	2.175 (±0.346)a	1.795 (±0.134)a	1.955 (±0.262)a	1.705 (±0.346)a
Arachidic acid C20	0.895 (±0.007)b	0.805 (±0.077)b	0.870 (±0.169)b	1.660 (±0.399)a	1.185 (±0.106)b

●Values are means ± SD of three replicates
●Means not sharing a common following letter in a raw are significantly different at $p<0.05$

cultures for the values of C8, C10, C14, C15, C16, C16-1, C18-1, C18-2, and C18-3 in the fermented milk. The values of stearic acid (C18) and arachidic acid (C20) were significantly ($P<0.05$) higher in the fat of milk fermented by *Streptococcus thermophilus* than those fermented by the other cultures whereas lauric acid (C12) value was high in that one fermented by *Lactococcus lactis*. Considering the data in Table (5), the present results indicate that a majority of the fatty acids were not affected by starter cultures fermentation except the levels of myristic acid, oleic acid and palmitic acid which increased while palmitoleic acid and arachidic acid decreased in the fermented camel milk as compared with unfermented milk (Table 5). In the same trend Rao and Reddy (1984) found that fermentation of whole milk by *Lactobacillus acidophilus, L bulgaricus,* and *Streptococcus thermophilus* resulted in a moderate but significant increase in the levels of saturated fatty acids and oleic acid with a concomitant decrease in the levels of linoleic and linolenic acids.

This finding is in agreement with Alm (1982a) who found small differences between unfermented and fermented milk products for the relative composition of fat and the fatty acid profile. Oberman (1985)

found that the lipase activity of lactic acid bacteria influenced the changes in fatty acid pattern.

4.2.5. Vitamins (C, B1 , B2) of fermented camel milk

The vitamin C, riboflavin and thiamine contents of camel milk fermented at 43ºC for 6 hours by five starter cultures are shown in Table (10). The vitamin C content was 3.66, 5.55, 7.42, 7.35, 7.34 and thiamine content was 0.295, 0.291, 0.286, 0.285, 0.280 while that of riboflavin content was 0.352, 0.361, 0.368, 0.384, 0.343, respectively for camel milk fermented by *L. acidophilus, L. bulgaricus, Latococcus lactis*, *St. thermophilus* and mixed cultures of *L. bulgaricus* and *St. thermophilus* (1:1).

The present data indicate that vitamin C content of the pasteurized camel milk compared with fresh milk decreased significantly ($P<0.05$) (Table 3). After fermentation at 43ºC for 6 hours by five starter cultures vitamin C decreased significantly whereas riboflavin and thiamine Contents showed minor decreases (Table 10). This finding agrees with those obtained by Oberman (1985) who found that lactic acid bacteria resulted in a decrease of about 50% in vitamin B6, B12 and vitamin C level, while only small

Table (10) : Vitamin C, thiamine and riboflavin content (mg/kg) of camel milk fermented for 6 hours at 43ºC by selected starter cultures

Vitamins (mg/kg)	*Lactobacillus acidophilus*	*Lactobacillus bulgaricus*	*Lactococcus lactis*	*Streptococcus thermophilus*	Yogurt culture (1:1) *St. thermophilus* and *L. bulgaricus*
Vitamin C	7.42 (±0.077)a	3.66 (±0.036)c	7.35 (±0.023)a	7.34 (±0.031)a	5.55 (±0.036)b
Thiamine (B1)	0.280 (± 0.077)a	0.285 (±0.007)a	0.295 (±0.006)a	0.286 (±0.002)a	0.291 (±0.007)a
Riboflavin (B2)	0.352 (±0.022)a	0.361 (±0.009)a	0.368 (±0.008)a	0.364± (0.004)a	0.343 (±0.001)a

- Values are means ± SD of three replicates
- Means not sharing a common following letter in a raw are significantly different at

  $P < 0.05$

changes in vitamin A, B1, B2 and niacin took place. On the other hand, Baranova *et al*. (1998) reported that fermentation of goat milk by selected lactic acid bacteria did not influence tocopherol contents, but there was a slight decrease in thiamine and riboflavin content, and a significant decrease in vitamin C ($P< 0.05$). Similarly Alm (1982c) reported that fermented milk products in general showed an increase in folic acid content and a slight decrease in vitamin B12 while other B-vitamins were affected only slightly in comparison to raw milk. In the same trend, Saidi and Warthesen (1993) observed that yogurt fermentation carried out in the laboratory for 5 h reduced the riboflavin content.

In other way, Bonczar and Regula (2003) found that the vitamin C and ascorbic acid contents decreased in ewe's milk after pasteurization and in yogurts during storage period, but increased with increasing amount of starter culture. In contrast, Hartman and Dryden (1965) think that several cultures are capable of synthesizing certain vitamins: for example, some strains of lactic bacteria have been found to augment the vitamin B12 content of the product by 20-30% or more. In another study, Khamagaeva *et al*. (1986) found an increase in the content of thiamin and riboflavin by 27 and 18%, respectively,

when the milk was inoculated with the combination of starter micro-flora, bifidobacteria, *Lactobacillus bulgaricus* and kefir starter at a ratio of 1: 0.5: 0.5 in fermented milk products.

The decrease of vitamin C was higher in milk fermented by *L. bulgaricus* (3.66 mg/kg) followed by mixed culture (1:1) of *L. bulgaricus* and *St. thermophilus* (5.55 mg/kg), *St. thermophilus* (7.34 mg/kg) *Lactococcus lactis* (7.35 mg/kg), and *L. acidophilus* (7.42 mg/kg), whereas no significant differences were observed in the riboflavin and thiamine content of fermented camel milk between the five starter cultures (Table 10).

On the other hand, many authors found that during fermentation the lactic acid bacteria require vitamins for growth, and certain micro-organisms produce vitamins at a higher rate than others do. The changes in the vitamin content are dependent on the kind of microorganisms and on the time and temperature of incubation. Laxminarayana and Shankar (1980) concluded that the vitamin content can be increased with selected cultures.

4.2.6. Amino acid composition of fermented camel milk

The amino acid composition of camel milk fermented by the five

starter cultures is presented with the recommended FAO requirements for pre-school children (2-5 years) Table (11).

The contents of essential amino acids such as valine, threonine, methionine, isoluecine, leucine, histidine, lysine and (phenylalanine + tyrosine) in the fermented camel milk were found to be higher than those of the FAO/WHO/UNU (1985). These findings confirm the excellent nutritional quality of fermented camel milk protein.

The values of amino acids in this work are much higher than those given by Rao *et al*. (1987) who studied the amino acid of Labneh (a concentrated yogurt product consumed routinely in the middle East) made from goat and cow milk. This variation in the amino acid composition may be due to differences in preparation procedure, source of milk (Goat or cow) and the type of final product. Referring to Table (11), the values of glutamic, proline, leucine, lysine and aspartic acid were higher compared to other amino acids in the five types of fermented camel milk products. The results show that there are no significant differences ($P>0.05$)in values of the individual amino acids between the five cultures except for phenylalanine which was lower in milk fermented by *Lactococcus lactis* (3.38%) and

Table (11) : Amino acids profile (g/100g) of camel milk fermented for 6 hours at 43ºC by selected starter cultures

Amino acids (g/100g)	*Lactobacillus acidophilus*	*Lactobacillus bulgaricus*	*Lactococcus lactis*	*Streptococcus thermophilus*	Yogurt culture (1:1) *S.thermophilus* and *L. bulgaricus*	FAO / WHO reference value*
ASP	6.96 (±1.44)a	7.84 (±0.52)a	7.85 (±0.11)a	6.99 (±0.16)a	7.85 (±0.23)a	
THR	4.62 (± 0.95)a	5.25 (±0.45)a	5.12 (±0.07)a	4.62 (±0.17)a	5.20 (±0.13)a	3.4
SER	4.14 (±0.83)a	4.68 (±0.31)a	4.75 (±0.13)a	4.22± (0.19)a	4.66 (±0.03)a	
GLU	17.02 (±3.09)a	19.24 (±1.21)a	19.44 (±0.46)a	17.22 (±0.52)a	19.39 (±0.47)a	
PRO	10.95(±0.12)a	10.88 (±0.33)a	11.28 (±0.11)a	10.22 (±0.15)b	11.17 (±0.21)a	
GLY	1.68 (±0.37)a	1.88 (±0.11)a	1.86 (±0.02)a	1.68 (±0.03)a	1.91 (±0.11)a	
ALA	2.83 (±0.71)a	3.24 (±0.17)a	3.14 (±0.04)a	2.82 (±0.05)a	3.21 (±0.20)a	
VAL	5.54 (±1.16)a	6.18 (±0.31)a	6.02 (±0.09)a	5.80 (±0.12)a	6.54 (±0.33)a	3.5
METH	2.54 (±0.58a	2.87 (±0.23)a	2.82 (±0.02)a	2.62 (±0.06)a	2.88 (±0.05)a	(M+C) 2.5**
ILEU	4.78 (±0.97)a	5.33 (±0.29)a	5.08 (±0.05)a	4.88 (±0.16)a	5.69 (±0.00)a	2.8
LEU	8.86 (±1.87)a	10.09 (±0.64)a	9.90 (±0.11)a	9.01 (±0.24)a	10.20 (±0.50)a	6.6
TYR	3.32 (±0.79)a	3.75 (±0.23)a	3.65 (±0.08)a	3.41 (±0.23)a	3.34 (±0.13)a	
PHY	4.57 (±1.03)a	3.96 (±0.63)a	3.38 (±0.02)b	4.35 (±0.11)a	4.89 (±0.18)a	6.3
HIS	2.79 (±0.64)a	3.40 (±0.51)a	3.42 (±0.05)a	3.29 (±0.04)a	3.78 (±0.37)a	1.9
LYS	7.55 (±1.69)a	8.22 (±0.00)a	7.61 (±0.11)a	7.62 (±0.26)a	8.28 (±0.53)a	5.8
ARG	3.66 (±0.79)a	4.06 (±0.25)a	4.90 (±1.31)a	4.46 (±0.27)a	4.71 (±0.24)a	

● Values are means ± SD of three replicates
* Amino acid requirements patterns as suggested by FAO / WHO /UNU (1985) for pre-school children(2-5 years)
** Methionine + Cysteine
● Means not sharing a common following letter in a raw are significantly different at $p<0.05$

proline (10.22%) was lower in milk fermented by *Streptococcus thermophilus*.

Results in Table 11 indicate that concentrations of most of the amino acids were slightly increased due to fermentation. However, there were slight decreases in Valine, methionine and tyrosine values when compared to the concentrations of amino acids of unfermented milk in Table 4. In the same trend, Muradyan *et al*. (1976) reported that fermentation of milk by thermophilic lactic streptococci or acidophilic rods enriched the final products with at least 4 amino acids (cysteine, valine, proline and arginine).

4.2.7. Available sugars in fermented camel milk

The lactose, glucose and galactose content of fermented camel milk are presented in Table (12).

Since not much literature is available on the sugars content of fermented camel milk, comparisons will be made with results of researchers who studied fermented milks from other animals.

After fermentation of camel milk for 6h at 43°C, the lactose content was 3.75, 3.45, 3.04, 2.85, 2.86% and glucose content was 0.268, 0.155, 0.297, 0.276, 0.422%, while that of galactose content was 0.82, 0.59, 0.083, 0.119, 0.824%, respectively for camel milk

Table (12) : Lactose, Glucose and Galactose concentrations (%) of camel milk fermented for 6 hours at 43ºC by selected starter cultures

Sugars%	*Lactobacillus acidophilus*	*Lactobacillus bulgaricus*	*Lactococcus lactis*	*Streptococcus thermophilus*	Yogurt culture *
Lactose	3.45 ±0.098[b]	2.85 ±0.034[d]	3.75 ±0.108a[a]	3.04 ±0.111[c]	2.86±1.009[d]
Glucose	0.268 ±0.008[a]	0.155 ±0.042[a]	0.297 ±0.286[a]	0.276 ±0.016[a]	0.422 ±0.016[a]
Galactose	0.820 ±0.056[a]	0.59 ±0.064[b]	0.083 ±0.033[c]	0.119±0.056[c]	0.824 ±0.084[a]

- Values are means ± SD of three replicates

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

- Means not sharing a common following letter in a raw are significantly different at $p<0.05$

fermented by *L. acidophilus, L. bulgaricus, Lactococcus lactis*, *St. thermophilus* and mixed strains of *L. bulgaricus* and *St. thermophilus* (1:1). The residual lactose concentration in fermented milk by *L. bulgaricus* (2.85 %) was equal to that fermented by mixed strains of *L. bulgaricus* and *St. thermophilus* (1:1) (2.86%), but both were lower than those fermented by *St. thermophilus* (3.04%), *L. acidophilus* (3.45%) and *Lactococcus lactis* (3.75%). The variation in the hydrolysis amount of lactose may be due to the strains of lactic acid bacteria used and the growth temperature. The present results indicated that there is no significant difference was observed between the five cultures for the residual glucose while the residual galactose was similar for *L. acidophilus* (0.82%), and mixed yogurt cultures (0.82%), but it is slightly lower for *L. bulgaricus* (0.59%) and lower in milk fermented by *St. thermophilus* (0.083%) than those fermented by the other strains. These results are in accordance with that reported by Toba *et al*. (1983) who found decrease in lactose content from 6.53 to 4.22% and increase in glucose and galactose in yogurt prepared by fermentation with *L. bulgaricus* and *St. thermophilus*. Also, Brein (1999) studied the sugar profile of cultured dairy products in UK, and confirmed that the most lactic acid fermentations result in a decrease

in lactose and increase in galatose. In the same trend, larger decreases in the lactose content brought about by the culture bacteria have been observed during the storage of yogurt (Alm, 1982b). In agreement with the present finding Saitmuratova and Sulaimanova (2000) found that the carbohydrates content of *Shubat* (fermented camel milk) was lower 3-5 times than those of unfermented camel milk.

4.2.8. Organic acids and ethanol contents in fermented camel milk

The lactic acid, formic acid, acetic acid and ethanol concentrations in the fermented camel milk products are shown in Table (13).

Three organic acids (lactic acid, formic acid and acetic acid) were detected while ethanol was not detected in final fermented camel milk products. The concentration of lactic acid were 0.6, 0.73, 0.23, 0.47, 0.85% and those of formic acid were 0.024, 0.026, 0.014, 0.026, 0.031%, while those of acetic acid were 0.021, 0.025, 0.009, 0.020, 0.025% respectively, for fermented camel milk for 6h at 43ºC by *L. acidophilus, L. bulgaricus, Lactococcus lactis*, *St. thermophilus* and mixed strains of *L. bulgaricus* and *St. thermophilus* (1:1).

Organic acids are produced during the metabolism of fermentable sugars. Lactic, acetic and propionic acids are formed

Table (13): Organic acids and Ethanol concentration (%) of camel milk fermented for 6 hours at 43ºC by selected starter cultures

Chemical components	*Lactobacillus acidophilus*	*Lactobacillus bulgaricus*	*Lactococcus lactis*	*Streptococcus thermophilus*	Yogurt culture*
Lactic acid	0.6 ± 0.028^{c}	0.73 ± 0.028^{b}	0.23 ± 0.014^{e}	0.47 ± 0.014^{d}	0.85 ± 0.014^{a}
Formic acid	0.024 ± 0.028^{a}	0.026 ± 0.001^{a}	0.014 ± 0.002^{b}	0.026 ± 0.001^{a}	0.031 ± 0.001^{a}
Acetic acid	0.021 ± 0.001^{a}	0.025 ± 0.001^{a}	0.009 ± 0.001^{b}	0.020 ± 0.001^{a}	0.025 ± 0.001^{a}
Ethanol	ND	ND	ND	ND	ND

● Values are means ± SD of three replicates.
ND = Not Detected.
*Combination of *St.thermophilus and L bulgaricus* 1:1.
● Means not sharing a common following letter in a raw are significantly different at $p<0.05$

during lactic fermentation (Rasic and Kurman, 1978). In this study the lactic acid concentration was higher in milk fermented by yogurt culture (0.85%) followed by that of *L bulgaricus* (0.73%), *L. acidophilus* (0.6%) *St. thermophilus* (0.47%), and *Lactococcus lactis* (0.23%). The concentration of formic acid (0.014%) and acetic acid (0.009%) in milk fermented by *Lactococcus lactis* were lower than those fermented by the other cultures while no significant differences ($p>0.05$) observed between the other cultures.

Formation of volatile acids during fermentation of Swedish fermented milk products showed that acetic acid and ethanol were low in yogurt than in bifidus milk (Alm, 1981). During fermentation of skim milk with lactic acid bacteria, seven organic acids were detected by Kato *et al*. (1992). In another study Damir *et al*. (1992) found more than six organic acids during kishk fermentation, lactic acid was the highest while formic acid the lowest.

4.2.9. Sensory evaluation of fermented camel milk products

Samples of camel milk fermented for 6h at 43ºC by selected starter cultures were sensory evaluated by 10 untrained panelists for color, smell, consistency, taste and overall acceptability. The mean sensory evaluation scores are summarized in Table (14).

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 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 tasters preferred fermented camel milk made by yogurt starter culture followed by *L. bulgaricus, L. acidophilus*, *St. thermophilus* and *Lactococcus lactis*. On the other hand, 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 *Lactococcus lactis* was the least. Camel milk fermented by yogurt

Table (14): Summary of sensory evaluation scores of fermented camel milk products

Attribute	Camel milk fermented for 6h at 43C by selected 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]	7.3(±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 ; 7 = good ; 5=acceptable, 3 = poor ; 1 = extremely poor).
* Combination of *St.thermophilus and L. bulgaricus* 1:1.

culture at 43ºC for 6h was considered the better product and presented good scores except for consistency. However, the consistency of all fermented camel milk products was watery and indicated a fragile and heterogeneous structure.

This observation agreed with Abou-Tarboush (1994) who reported that acidophilus milk made from camel milk was watery and precipitated in the form of flocs. The results presented by Attia *et al*. (2001) showed that the fermentation of camel milk by starter culture did not reveal curd formation. In another study, the fermentation of camel and cow milk by lactic acid bacteria indicated that the cultures were less active in camel milk than cow milk while camel milk failed to reach a gel-like structure after 18h incubation, the author attributed that to the presence of growth inhibitors in camel milk (Gran *et al*, 1991).

On the other hand, Farah (1990) reported that the Susa (Traditional fermented camel) can be improved by using selective mesophilic lactic acid culture.

On the other hand, making cheese and other fermented products from camel milk is difficult and complicated. Previous studies indicate that raw camel milk contains several antimicrobial agents such as high

levels of lysozyme that can limit microbial growth than in milk from other domestic animals, (Barbour *et al*. 1984; El Agamy *et al*. 1992; Farah, 1993). The antimicrobial activity of other natural proteins such as lactoferrin, lactoperoxydase and immunoglobulins was studied (El-Agamy *et al*., 1992 and El-Agamy, 1994). Each of these antimicrobial agents possesses a selective spectrum of activity against specific strains of bacteria and viruses.

Other researchers found that camel showed poor rennetability and the curd formed was looser and weaker than curd from cow or goat milk (Bayoumi, 1990; Hafez and Hamzawi, 1991), others suggested for coagulation of camel milk high dosage of calf rennet (Gast *et al*., 1969; Chapman, 1985), while others advised to mix it with other milk such as cow or goat milk (Rao *et al*, 1970; Mehaia, 1993b).

5. Summary and Conclusion

During fermentation of camel milk using selected starter cultures of lactic acid bacteria , the microbiological analysis showed that the counts of the five starter cultures increased as fermentation time progressed and counts of *L.bulgaricus* were highest while, those of *Lactococcus lactis* were lowest. The final fermented milk products were free from pathogenic bacteria such as *Salmonella spp*., *Staphylococcus aureus*, *Listeria monocytogenes, E.coli O157:H7* and *Bacillus cereus,* while the total coliform and yeast and mold counts were less than 10 cfu per ml.

The biochemical investigation during fermentation of the camel milk indicated that the pH of camel milk fermented by single starter culture of *L. bulgaricus* was lower than those by other single cultures, whereas the combination of *L. bulgaricus* CH2 plus *S. thermophilus 37* (1:1) gave low pH and high acidity compared to the pure single starter cultures. The amount of free amino acid groups released after 6h of fermentation by the five starter cultures showed that the proteolytic activity of *L. bulgaricus* was the highest one compared to *S.thermophilus*, *L. acidophilus* and *Latococcus. lactis* but it was lower

than that released by mixed cultures of *L. bulgaricus* and *S. thermophilus* (1:1). The fatty acid profile of fermented milk showed that the palmitic acid was the major saturated fatty acid followed by myristic and stearic acid while oleic acid followed by linoleic acid were the major unsaturated fatty acids. The majority of the fatty acids were not affected by starter cultures fermentation except the levels of myristic acid, oleic acid and palmitic acid were increased while palmitoleic acid and arachidic acid were decreased in the fermented camel milk as compared with unfermented milk.

The values of amino acids were generally increased slightly due to fermentation. However, there was a slight decrease in valine, methionine and tyrosine when compared to those of unfermented milk. The contents of essential amino acids such as valine, threonine, methionine, isoluecine, leucine, histidine, lysine and (phenylalanine + tyrosine) in the fermented camel milk were found to be higher than those of the FAO/WHO/UNU (1985) recommended as reference pattern.

The residual lactose concentration in fermented milk by *L. bulgaricus* was equal with that fermented by mixed cultures of *L. bulgaricus* and *St. thermophilus* (1:1), but both were lower than those

fermented by *St. thermophilus*, *L. acidophilus* and *Lactococcus lactis*. There was no significant difference observed between the five starter cultures for the residual glucose while the residual galactose was similar for *L. acidophilus*, and mixed starter cultures, but, it was slightly lower for *L. bulgaricus* and much lower in milk fermented by *St.thermophilus* than those fermented by the other starter cultures.

The vitamin C content of the pasteurized camel milk decreased significantly ($P<0.05$) in all fermented milk products whereas riboflavin and thiamine content showed minor decreases. Three organic acids (lactic acid, formic acid and acetic acid) were detected while ethanol was not detected in fermented camel milk products.

The sensory evaluation of fermented camel milk showed that the camel milk fermented by yogurt culture at 43ºC for 6h considered the better product and had good scores for color, smell, taste and overall acceptability than those fermented by other single starter cultures. In general the consistency of fermented milk products was found to be watery and fragile indicating poor structure.

In conclusion camel milk fermented with mixed culture of *Lactobacillus bulgaricus* and *Streptococcus thermophilus* (1:1) at 43ºC for 6 hours was considered the better products, most acceptable

and present a good potential for commercial production compared to those fermented by other culture strains. In addition, the fermentation under control conditions gave high microbial quality of fermented camel milk products.

It can be concluded that the lactic acid bacteria *L. bulgaricus* and mixed cultures of *L. bulgaricus* and *St. thermophilus* (1:1) are the major vitamin C user in camel milk, whereas *L. acidophilus, Lactococcus lactis* and *St. thermophilus* did not use half the quantity of vitamin C in camel milk.

Furthermore work is recommended to study the possibility of using mixed starter cultures of lactic acid bacteria with yeasts. Also optimum conditions of inoculums size, incubation time and temperature for large number strains of lactic acid bacteria should be ascertained. On the other hand more research is needed to improve the consistency of the fermented camel milk products.

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