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RESEARCH ARTICLE

EFFECT OF FERMENTATION BY SELECTED LACTIC ACID BACTERIAON THE FATTY ACIDS OF CAMEL MILK FAT

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Abstract

The objectives of this research were to determine the fatty acids composition of pasteurized camel milk and to study the effect of fermentation by lactic acid strains of Lactobacillus acidophilus and mixed yogurt starter cultures (Streptococcus thermophilus &Lactobacillus bulgaricus (ratio of 1:1) on the fatty acids profile of pasteurized camel milk. Fresh pasteurized camel milk was inoculated with 5% of Lactobacillus acidophilus and mixed yogurt starter cultures (Streptococcus thermophilus &Lactobacillus bulgaricus (1:1) and incubated at 43°C in a circulating water bath for 6 hours. The major fatty acids of pasteurized camel milk were palmitic (26.5%) and oleic (24.2%) acids followed by stearic (12.2%), myristic (10.2%) and palmitoleic (9.98%). The short-chain fatty acids (C8–C10) were present in small amount. Fermentation process significantly increased the content of (saturated fatty acids) myristic acid, palmitic acid, and lauric acid, however, arachidic acid (C20) was significantly decreased. On the other hand, the mono-unsaturated fatty acid oleic (18:1) was increased but, the Palmitoleic (C16:1) was decreased significantly. The concentration of polyunsaturated fatty acids, linoleic (C18:2) and linoleic (C18:3) were increased in the fermented product compared to unfermented pasteurized milk. This study showed there are no significant differences (P>0.05) in the values of fatty acids composition among the two cultures used in the fermentation process.

Introduction:-

Camels belong to the family Camelidae and thereby to the sub-order Tylopoda. The Camelidae originated in North America where their earliest fossil remains have been found. Some of the camels migrated to the deserts and semi-deserts of northern Africa and the Middle East (Simpson, 1945; Zeuner, 1963). The majority of the world's camel population is of dromedary type except for a small population of Bactrian camels in central Asia. World Camel population is estimated to be around 25.89 million spread across 47 countries, Somalia has the highest population of 7.00 million followed by Sudan 4.25 million and Ethiopia 2.4 million camels (FAO STAT, 2011). Camels are considered to be a good source of milk and meat and are used for other purposes such as transportation and sports racing (Kaufmann, 2005). 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

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found in starter cultures of today include those bacteria that predominated in the historical 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. Farah et al. (1990) found that the consistency of fermented camel milk was thin and a precipitate in the form of flacks was formed rather than a coagulum after fermentation. The consistency of curd camel milk remains thin due to improper coagulum formation which might be due to small micelles size of the fat globules (Mal and Pathak, 2010). On the other hand, Mohamed et al, (1990) observed that camel milk failed gel-like structure after 18h incubation with lactic acid bacteria; this was attributed to the presence of antibacterial factors such as lysozymes, lactoferrin, and immunoglobulin in camel milk. The fatty acids are divided according to the linkage of the carbon atoms into saturated and unsaturated fatty acids. In saturated fatty acids, the carbon atoms are linked in a chain by single bonds, in unsaturated fatty acids by one or more double bonds. Higher content of long-chain fatty acids (C14-C18) and lower content of short-chain fatty acids (C4-C12) are present in camel milk compared to cow milk (Narmuratova et al. 2006). Some researchers claim that the value of camel milk is due to the high concentrations of polyunsaturated acids, especially, linoleic acid which is essential for human nutrition (Khan, and Iqbal.2001). Milk fat of dromedary camels carries a lower level of carotene and lesser concentrations of short-chain fatty acids as compared to the milk of bovine (Stahl et al., 2006). Milk fat is the most variable of all milk components and is recognized as a major contributor in determining the consumer acceptability of most dairy products (Day, 1960). 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&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. The aim of this research was to determine the fatty acid composition of camel milk fat and the effect of fermentation by selected starter cultures of lactic acid bacteria on the fatty acids composition of pasteurized camel milk.

Materials and Methods:-

Sources and maintenance of cultures:

Culture strains of Lactobacillus acidophilus**&** mixed yogurt culture (Streptococcus thermophilus and Lactobacillus bulgaricus (1:1) used in this study were obtained as lyophilized pure culture from Chr.Hansen'sLaboratorium. (Hørsholm, Denmark A/S).The preparation of the inoculum was performed with the activation of the culture in 100 mL of sterile 10% SM with weekly transfers. The purity of cultures was routinely checked by performing the Gram stain.

Preparation of fermented camel milk:

Fresh whole camel milk from Camelus dromedarius was obtained from a private herd. Milk was immediately cooled and kept at 5±1oC during transportation to the laboratory. The whole camel milk was pasteurized in 500-ml quantities at 80°C for 15min in a water bath and cooled immediately. The milk samples (500ml) were equilibrated for 1h at the fermentation temperature (43ºC) in a water path before inoculation with the starter cultures. Every 500 ml of pasteurized camel milk was inoculated with 5% of Lactobacillus acidophilus**&** mixed yogurt culture (S.thermophilus and L. bulgaricus (1:1),the contents were thoroughly mixed after inoculation and incubated at 43ºC in a circulating water bath for 6h. The final products of fermented camel milk after 6 hours of incubation were analysis for the fatty acids profile. The whole experimental procedure was repeated three times using three different batches of fresh camel milk. Triplicate determinations of fatty acids content were done for each batch.

Fatty acids analysis:

For fatty acid, analysis lipids were extracted from the camel milk samples by the Rose-Gottlieb cold extraction method (Pearson, 1977). 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 an 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 a 1.5ml methanolic KOH (0.5N) solution by refluxing for 10min at 85˚C. After the addition of 4ml boron trifluoride methanol complex reagent (20% BF3 in methanol), the sample was boiled for another 5 min. The FAMEs were thrice extracted from the 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. 3M x 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 4^{o}C /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 / cm2. 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 was used as an internal standard. The fatty acid profile was quantitated according to procedures outlined in AOCS (1977).

Results and Discussion:-

Fatty acid composition of pasteurized camel milk:

The fatty acids of pasteurized camel milk fat were shown in Fig-1. Results of this study indicated that palmitic concentration (26.5%), and oleic (24.2%) acids were the major fatty acids found followed by stearic (12.2%), myristic (10.2%) and palmitoleic (9.98%) acids. The results in the present study are similar to those reported by Abu-Lehia (1987) and comparable to those found by Sawaya et al. (1984a) and Gorban & Izzeldin (2001), but it was lower than that reported by Yagil. (1982). Our results revealed that the short-chain fatty acids (C8–C12) were present in small amount while the concentrations of C14:0, C16:0 and C18:0 are relatively high, which are in accordance with 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). On the other hand, other researchers confirmed that camel milk fat has a higher content of long-chain fatty acids and lower content of short-chain fatty acids as compared to cow milk (Narmuratova et al., 2006; Stahl et al., 2006). These results were similar to those obtained by (Konuspayeva et al., 2008) who found that the fatty acid composition of camel milk fat differed from mammalian fats by its high content of the long-chain fatty acids C14:0, C16:0, and C18:0. Fatty acids composition is influenced by environment and physiological factors such as nutrition, stage of lactation and genetic differences within the species (Farah et al., 1989).

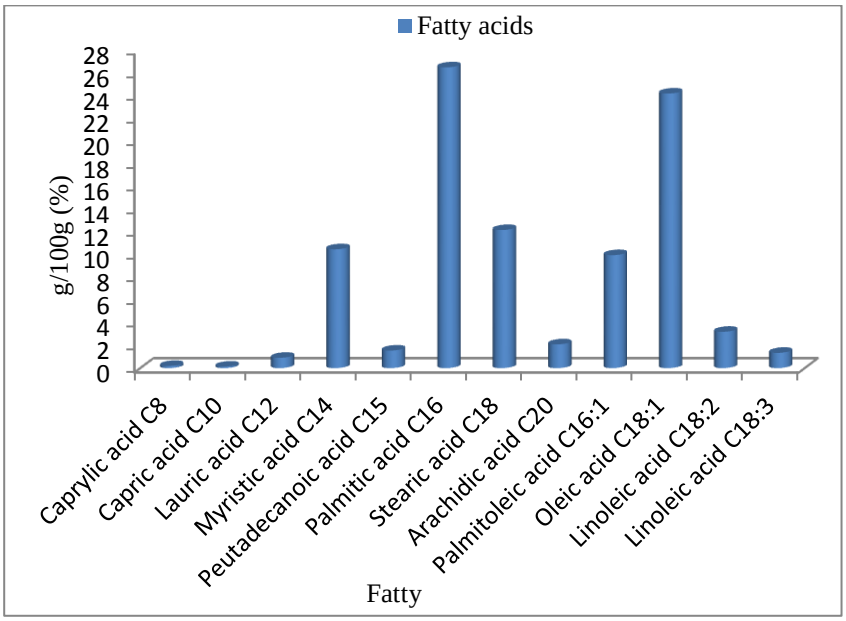

Fig. 1:- Fatty acid% composition of pasteurized camel milk fat.

Fatty acid composition of fermented camel milk:

Fig. 2 shows the values of saturated Fatty acids (g/100g) of pasteurized camel milk fat and fermented products. In the present study saturated fatty acids were the most predominant fatty acids in fermented camel milk. The level of palmitic acid (42.98%) was the major saturated fatty acid followed by stearic acid (12.2%), meristic (10.5%) and pentadecanoic acid (1.55%). These results agree with those reported by Guler et al. (2010) and Prandini et al. (2007) who reported that palmitic acid was the major saturated fatty acid in fermented milk.

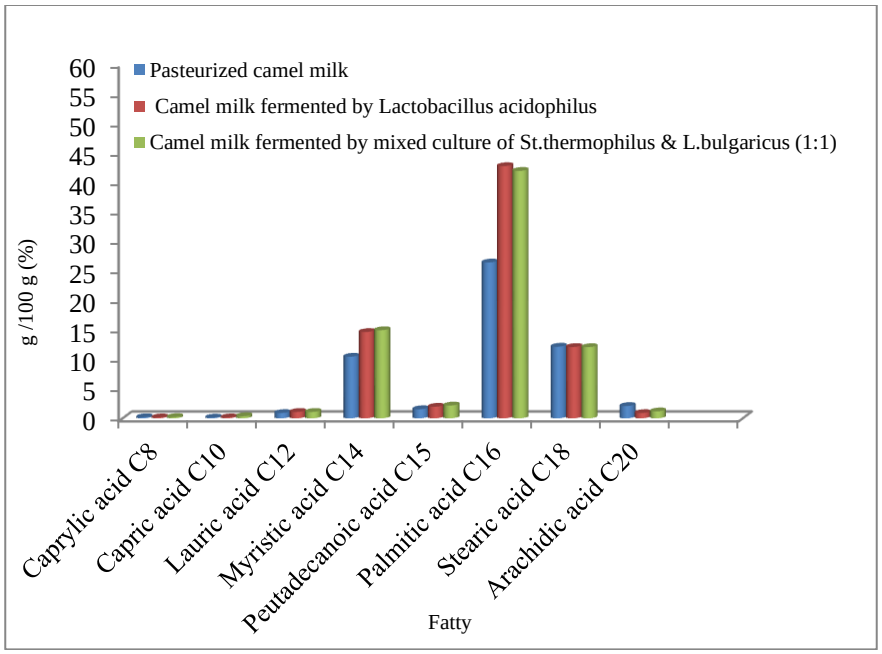

Fig. 2:- Saturated fatty acids% (g/100g) of pasteurized camel milk fat and fermented products by selected starter cultures.

Palmitic acid (C16) of pasteurized camel milk fat increased from 26.5% to 42.79% in camel milk fermented by L. acidophilus, while fermented by mixed culture of St.thermophilus&L. bulgricus 1:1, increased to 41.98%. Meristic acid increased from 10.5% in pasteurized milk to 14.71% and 15.02% respectively, whereas psentadecanoic fatty acid increased from 1.55% in pasteurized milk to 1.97 and 2.19% for fermented camel milk by L. acidophilus and mixed yogurt culture respectively. Cultures we used in the present study had only a minor effect on the values of caprylic acid (C8), Capric acid (C10), lauric acid (C12) and stearic acid (C18) of pasteurized camel milk. The values of arachidic acid (C20) were significantly ($P<0.05$) decreased in the fermented camel milk by L. acidophilus and mixed yogurt culture. In our study palmitic acid was the most abundant fatty acid in fermented milk products of both cultures (42.79% and 41.98%). This finding agrees with Prandini et al (2007) who reported that palmitic acid (31.01%) was a major fatty acid in fermented milk. On the other hand, Bahobail et al (2014) observed an increase in fatty acids concentration of raw camel milk after subjected to the fermentation process. Yadav et al. (2007) reported that the addition of probiotic bacteria to Dahi (Indian fermented milk product) increased saturated fatty acid content of fermented milk in comparison to the control unfermented sample. This study showed there are no significant differences ($P>0.05$) in the values of the saturated fatty acids between the two cultures used in the fermentation of pasteurized camel milk.

Fig. 3 shows the mono-unsaturated fatty acids composition of pasteurized camel milk and its corresponding products, which were fermented products by adding Lactobacillus acidophilus and mixed cultures of St.thermophilus&L.bulgricus ratio of 1:1.The oleic acid (18:1) followed by palmitoleic (16:1) were the major monounsaturated fatty acid in pasteurized camel milk and fermented products. On the other hand, oleic (18:1) increased from 24.2% in pasteurized milk to 29.42% in fermented milk products by Lactobacillus acidophilus and to 29.11% in fermented milk by mixed yogurt cultures of St.thermophilus &L.bulgricus ratio of 1:1. Palmitoleic (C16:1) decreased from 9.98% in pasteurized milk to 1.56% and 2.19% in fermented milk by Lactobacillus acidophilus and by mixed yogurt cultures of St. thermophilus& L.bulgricus 1:1 respectively.

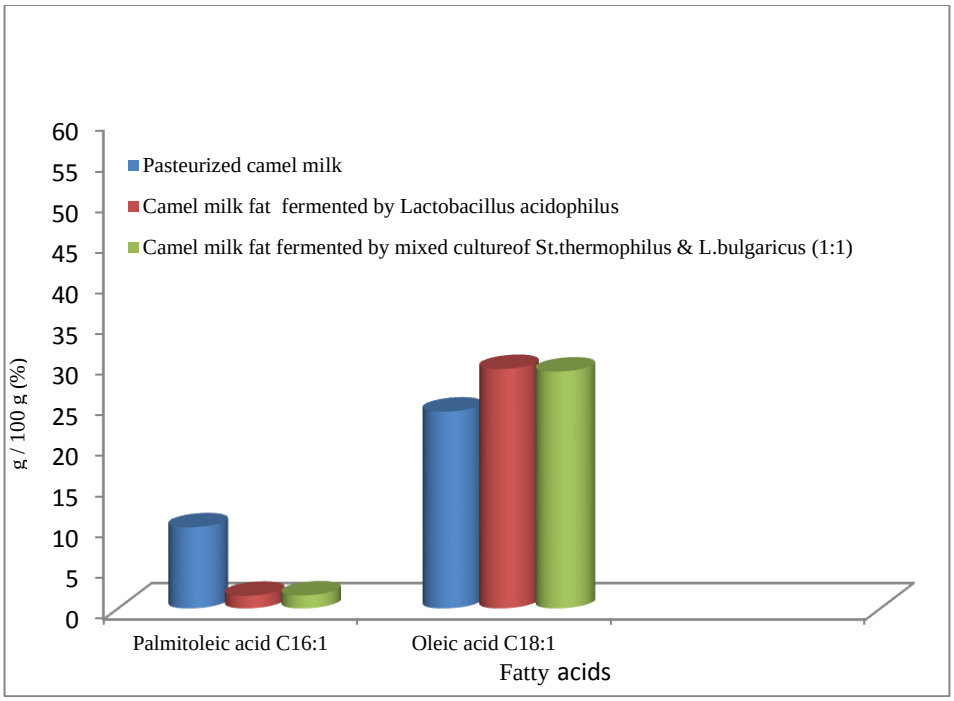

Fig. 3:- Mono-unsaturated fatty acids% (g/100g) of pasteurized camel milk fat and fermented products by selected starter cultures.

The increase of oleic (18:1) in fermented camel milk agrees with that reported by Sumarmono et al. (2015) in yogurt of fresh goat milk, whereas Salomon et al. (2009) found that the cultures commonly used in production of sour milk (Sana, yogurt) had a minor effect on fatty acid composition of milk. Ersan (2013) demonstrated that probiotic bacteria could improve the fatty acid profile of fermented creams.

The poly-unsaturated fatty acids (C18:2&C18:3) of pasteurized camel milk and fermented products by selected starter cultures is given in Figure 4. The major polyunsaturated fatty acids include linoleic acid C18:2 and linoleic acid C18:3. Linoleic C18:2 increased from 3.21% in pasteurized camel milk to 3.44% and 3.43% while linoleic 18:3 increased from 1.35% in pasteurized milk to 1.87% and 1.71% in camel milk fermented by lactobacillus acidophilus and mixed yogurt cultures of St. thermophilus&L. bulgricus ratio of 1:1 respectively. In contrary to this finding, Gerchev,and Mihaylova(2012) reported, no significant changes observed in content of fatty acids of fermented milk (yogurt) made from raw sheep milk.

These results indicated that there are no significant differences (P>0.05) in the values of poly-unsaturated fatty acids among the two cultures used in the fermentation process of pasteurized camel milk. It can be concluded the differences in the fatty acids profiles of pasteurized camel milk and its fermented products by selected starter cultures were due to the lactic acid activities. Lipolysis is one of the main biochemical changes significantly affecting the characteristics and shelf life of many dairy products (Senel et al.2010). In previous study, Adam A.I et al (2016) reported in controlled fermented camel milk, fatty acids classes of fermented camel milk have various trends affected by starter cultures fermentation.

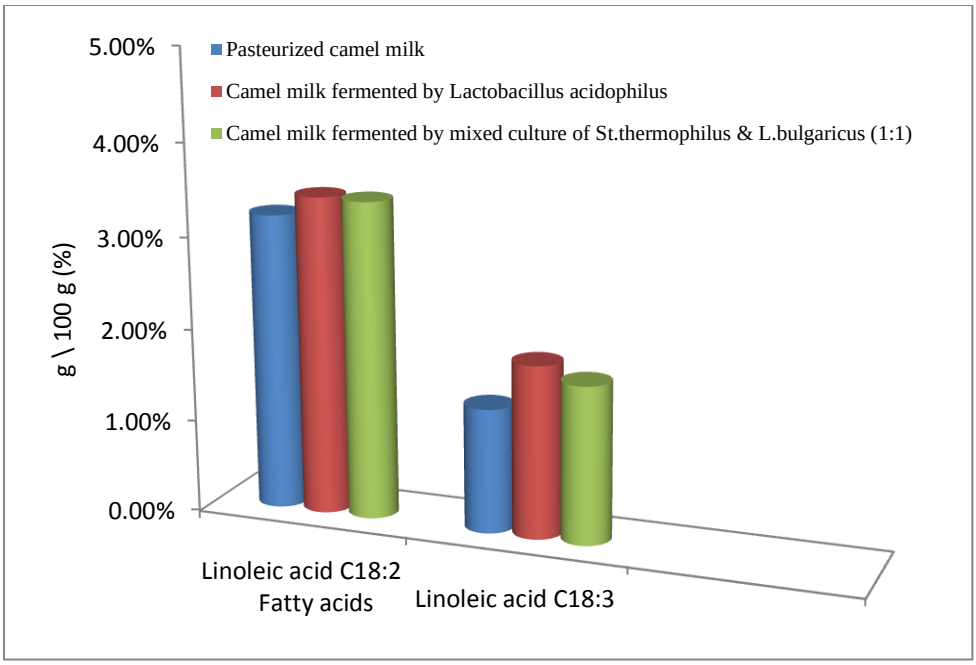

Fig. 4:- Poly-unsaturated fatty acids% (g/100g) of pasteurized camel milk fat and fermented products by selected starter cultures.

Conclusion:-

The major fatty acids of pasteurized camel milk were palmitic (26.5%) and oleic (24.2%) acids followed by stearic (12.2%), myristic (10.2%) and palmitoleic (9.98%). The short-chain fatty acids (C8–C10) were present in small amount. Fermentation process significantly increased the content of (saturated fatty acids) myristic acid, palmitic acid, and lauric acid, however, arachidic acid (C20) was significantly decreased. Mono-unsaturated fatty acid oleic (18:1) was increased but, the Palmitoleic (C16:1) was decreased. The concentration of polyunsaturated fatty acids, linoleic (C18:2) and linoleic (C18:3) were increased in the fermented product compared to unfermented pasteurized milk. There are no significant differences (P>0.05) in the values of fatty acids composition among the two cultures used in the fermentation process.

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