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EVALUATION OF MICROBIOLOGICAL QUALITY OF WHITE SOFT CHEESE MANUFACTURED FROM CAMEL AND SHEEP MILK

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Abstract
This study aimed to investigate microbiological quality of soft cheese made from camels', sheep and their mixtures milk. Cheeses were made using Camifloc enzyme as a coagulant after addition of calcium chloride. Camel's milk was mixed with sheep's milk at the levels of 25%, 50% and 75%, pure camel and pure sheep milk were also included. Then, the cheeses were stored for 21 days in the whey at room temperature (37° C - 40° C). Means of total bacterial count for cheeses produced from camel's milk, camel's milk: sheep's milk (75%: 25%), camel's milk: sheep's milk (50%:50%), camel's milk: sheep's milk (25%: 75%) and sheep's milk were 9.44×10^5, 16.75×10^5, 15.88×10^5, 18.33×10^5 and 22.11×10^5 (cfu/gm), respectively. The coliform bacterial count revealed 8.73×10^5, 7.94×10^5, 9.05×10^5, 8.94×10^5 and 7.68×10^5, respectively and he means of yeast and mould counts were 5.44×10^5, 7.05×10^5, 7.71×10^5, 5.58×10^5 and 6.57×10^5 (cfu/gm), respectively.
The psychotrophic bacterial count was found as 8.69×10^5, 9.43×10^5, 8.85×10^5, 8.94×10^5 and 9.07×10^5 (cfu/gm), respectively, Moreover there were significant ($P<0.05$) differences in total bacterial, coliform and yeast and mould counts during the storage period for all types of cheeses except sheep's milk cheese. However, there were non significant ($P>0.05$) differences during the storage period in the counts of psychrotrophic bacteria.
As camel cheese properties were improved by fortification of sheep milk, the present study recommended production of clean milk from camel and sheep in order to obtain good quality products

Keywords: soft cheese, camel and sheep milk, Camifloc, microbiological quality

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1.INTRODUCTION

Difficulties in making camel cheese include longer coagulation time, weak coagulum (Mehaia, 1993; Ramet, 2001) and the technique which is being used (Farah and Bachmann, 1987; Inayat et al., 2003 and El Zubeir and Jabreel, 2008).

Nour et al. (1979) found that the level of acid production by *Streptococcus lactics*, *S. faecalis* and *Lactobacillus bulgaricus,* were reduced when 2% NaCl was added, in making cheese from camel's milk. However Ahmed and El Zubeir (2011) suggested that the level of salting should be reduced when adding calcium chloride or further drying and storage of cheese should be done as some of panelists, reported that level of salting is more than they like.

Milk used for cheese making must be of good microbiological quality, total plate count less than10^4 cfu/ml and somatic cell count less than10^5 cfu/ml, in addition to the absence of pathogenic bacteria, antibiotics and inhibitors (Skeie, 2007). According to Benkerroum (2010), the microbiological results of camel milk cheese indicate that the cheese obtained has an acceptable hygienic quality as suggested by the absence of faecal coliforms, faecal streptococci, *Salmonella spp*. and clostridial spores. Furthermore, the inherent antimicrobial activity of camel milk could concur with the highly competitive nature of the lactic acid bacteria of the starter culture to limit the growth of undesirable microorganisms during the fermentation. Such an assumption could be supported by the relatively low total aerobic count in camel cheese compared to that obtained from cow or goat milk which normally exceeds 7 log units (Marino et al., 2000). Nonetheless the fact that cheese samples could be kept at 7–9° C for 7 days without microbial alteration (Ramet, 2001 and El Zubeir and Jabreel, 2008). Moreover pasteurization of camel milk before its

fermentation was found to improve the microbial content and to increase the shelf life of the product (Hassan et al., 2006).
The present study was carried out to assess the microbial content of cheese made from camels, sheep milk and their mixtures (25%, 50% and 75%) during the storage period on the cheese properties.

2. MATERIALS AND METHODS

Source of milk, Camifloc and salt

The present study was conducted during the period of June to September 2009. Fresh camel's milk was obtained from Camel Research Center, University of Khartoum, while fresh sheep's milk was obtained from a private farm in Khartoum North, Khartoum State, Camifloc powder (Bio Serae Laboratories, Bram, France) was obtained from the Department of Animal Production, Ministry of Animal Resources. Calcium chloride is a product of Merck (Denmark). The salt (commercial grade) was obtained from the local market.

Cheese processing

Five types of milk were used; camel's milk, sheep's milk and a mixture of camel's and sheep's milk 25%, 50% and 75%. Cheeses were made from the five types of milk under the same conditions using Camifloc enzyme, calcium chloride and sodium chloride. The cheeses were made using the available cheese making equipment. The milks were first filtered using clean white cheese cloth, and then the milks were heated to 62°C for15 minutes and then cooled for 5 minutes in an ice water. Then the cheese was made according to the Camifloc manufacture's instructions as was described previously by El Zubeir and Jabreel (2008).

Microbiological examination

The camel and sheep cheese samples were examined for total bacterial count, yeasts and moulds count, coliform count and psychotrophic bacterial count at 1, 3, 6, 15, and 21 day intervals.

The glass wares such as Petri dishes, test tubes, flasks, pipettes and bottles were sterilized in an oven at 170° C for two hours. Media, mixer, distilled water, and tips were sterilized by autoclaving for 15 minutes at 121° C (Marshall, 1992).
All media were obtained in a powder form and prepared according to the manufactures instructions. Plate count agar medium was used for determining the total bacterial count (Houghtby et al., 1992) and psychotrophic bacterial count (Frank et a.l., 1992). MacConkey agar medium was used to determine coliform count (Christen et al., 1992) and yeast extract agar was used for yeasts and moulds count (Frank et al., 1992).

Preparation of cheese samples

Ten grams of cheese was added to 90 ml of warm sterile distilled water and blended for two minutes. One ml of the dilution sample was transferred with sterile one ml graduated pipette to nine ml sterile distilled water in a test tube and mixed well. Using another sterile pipette, one ml of the latter dilution was transferred into a second dilution test tube. This process was repeated to make ten folds dilution from 10^{-1} to10^{5} (Marshall, 1992).
Suitable dilutions were used for the determination of total bacterial count, yeasts and moulds count, coliform count and psychotrophic bacterial count. The plates were inoculated in duplicates and left for two minutes to dry, and then were incubated at 32 ° C for 48 hours for total bacterial count and at 37° C for 24 hours for coliform counts. However psychotrophic count was incubated at 4° C for 7– 8 days and yeast and mould were incubated at 25° C for up to 72 hours. The plates were examined and the colonies were counted using a colony counter.

Statistical analysis

Data were analyzed using SPSS program (Statistical Package for Social Science, version 10.5) using Completely Randomized Design (CRD). The analysis was carried out by ANOVA test and Dunkan multiple ranges test to indicate the differences between means. Also

Chi-squire test was used to analyze sheet of panel tests.

3. RESULTS

Total bacterial count

Means of total bacterial count for cheeses produced from camel's milk, camel's milk: sheep's milk (75%: 25%), camel's milk: sheep's milk (50%:50%), camel's milk: sheep's milk (25%: 75%) and sheep's milk were 9.44×10^5, 16.75×10^5, 15.88×10^5, 18.33×10^5 and 22.11×10^5(cfu/gm), respectively as shown in Table 1. Non significant ($P>0.05$) differences were obtained for cheeses due to variations in types of milk except camel's milk. There were significant ($P<0.05$) differences in the total bacterial count for all types of cheeses during storage period except cheese made from sheep's milk (Table 2).

Table 1: Effect of type of milk on the microbiological properties of cheeses

PARAMETERS (cfu/gm)	Type of Cheese					L.S
	Camel's milk	Camel's milk: sheep's milk (75%: 25%)	Camel's milk: sheep's milk (50%:50%)	Camel's milk: sheep's milk (25%: 75%)	Sheep's milk	
	Means± SD	Means± SD	Means± SD	Means± SD	Means± SD	
Total bacterial count	$9.44\times10^5\pm101.90^b$	$16.75\times10^5\pm105.52^a$	$15.88\times10^5\pm112.55^a$	$18.33\times10^5\pm101.17^a$	$22.11\times10^5\pm308.83^a$	*
Coliform count	$8.73\times10^5\pm66.58^a$	$7.94\times10^5\pm55.12^a$	$9.05\times10^5\pm62.69^a$	$8.94\times10^5\pm60.66^a$	$7.68\times10^5\pm58.09^a$	NS
Yeast & mould count	$5.44\times10^5\pm46.71^a$	$7.05\times10^5\pm52.41^a$	$7.71\times10^5\pm55.00^a$	$5.58\times10^5\pm51.86^a$	$6.57\times10^5\pm52.60^a$	NS
Psychotrophic count	$8.69\times10^5\pm73.50^a$	$9.43\times10^5\pm72.20^a$	$8.85\times10^5\pm66.95^a$	$8.94\times10^5\pm70.30^a$	$9.07\times10^5\pm73.23^a$	NS

[a, b, c] :Values in the same row bearing different superscript letters are significantly different ($P < 0.05$)
L.S = Significant level N.S = Not significant * = ($P<0.05$) SD = Standard deviation

Table 2: Effect of the storage period on total bacterial count of cheeses made from camel's, sheep's and their mixtures milk

Types of cheeses	Storage period (days)					L.S
	1	3	6	15	21	
	Means± SD	Means± SD	Means± SD	Means± SD	Means± SD	
Camel's milk	$15.82\times10^5\pm88.61^a$	$14.88\times10^5\pm101.45^{ab}$	$12.22\times10^5\pm129.30^{ab}$	$10.05\times10^5\pm92.04^b$	$8.22\times10^5\pm67.11^b$	*
Camel's milk: sheep's milk (75%: 25%)	$19.92\times10^5\pm88.78^a$	$16.33\times10^5\pm94.20^{ab}$	$14.83\times10^5\pm126.69^{ab}$	$12.23\times10^5\pm89.53^b$	$9.12\times10^5\pm97.10^b$	*
Camel's milk: sheep's milk (50%:50%)	$22.12\times10^5\pm83.54^a$	$16.97\times10^5\pm106.63^b$	$15.76\times10^5\pm130.11^b$	$11.73\times10^5\pm82.78^b$	$10.26\times10^5\pm78.13^b$	*
Camel's milk: sheep's milk (25%: 75%)	$22.71\times10^5\pm112.5^a$	$20.59\times10^5\pm110.52^{ab}$	$18.64\times10^5\pm100.03^{ab}$	$18.08\times10^5\pm83.97^{ab}$	$11.61\times10^5\pm80.84^b$	*
Sheep's milk	$26.60\times10^5\pm86.26^a$	$23.12\times10^5\pm109.41^a$	$20.72\times10^5\pm130.62^a$	$20.46\times10^5\pm664.73^a$	$14.28\times10^5\pm100.24^a$	N.S

[a, b, c] :Values in the same row bearing different superscript letters are significantly different ($P < 0.05$)
L.S = Significant level N.S = Not significant * = ($P<0.05$) SD = Standard deviation

Table 3: Effect of the storage period on coliform count of cheeses made from camels, sheep and their mixtures milk

Types of cheeses	Storage period (days)					L.S
	1	3	6	15	21	
	Means± SD	Means± SD	Means± SD	Means± SD	Means± SD	
Camel's milk	$9.08\times10^5\pm 77.73^a$	$9.88\times10^5\pm75.83^a$	$5.03\times10^5\pm 70.77^b$	$5.25\times10^5\pm 43.45^{ab}$	$2.74\times10^5\pm 8.73^c$	*
Camel's milk: sheep's milk (75%: 25%)	$9.43\times10^5\pm 65.14^a$	$11.11\times10^5\pm 67.63^a$	$5.66\times10^5\pm 44.15^b$	$6.17\times10^5\pm 25.59^{ab}$	$3.21\times10^5\pm 36.37^b$	*
Camel's milk: sheep's milk (50%:50%)	$9.80\times10^5\pm 58.46^a$	$11.27\times10^5\pm 86.30^a$	$8.77\times10^5\pm 58.32^{ab}$	$7.12\times10^5\pm 53.11^b$	$4.17\times10^5\pm 37.56^b$	*
Camel's milk: sheep's milk (25%: 75%)	$10.69\times10^5\pm 52.72^{ab}$	$11.87\times10^5\pm 68.73^a$	$8.96\times10^5\pm65.65^{ab}$	$7.45\times10^5\pm 56.80^b$	$5.34\times10^5\pm 29.85^b$	*
Sheep's milk	$11.80\times10^5\pm 51.22^a$	$13.07\times10^5\pm 56.89^a$	$10.15\times10^5\pm 75.49^a$	$7.98\times10^5\pm 55.60^a$	$6.89\times10^5\pm 35.10^a$	N.S

[a, b, c] :Values in the same row bearing different superscript letters are significantly different ($P < 0.05$)
L.S = Significant level N.S = Not significant * = ($P<0.05$) SD = Standard deviation

Coliform count

The means of coliform bacteria count for cheeses from camel's milk, camel's milk: sheep's milk (75%: 25%), camel's milk: sheep's milk (50%:50%), camel's milk: sheep's milk (25%: 75%) and sheep's milk were 8.73×10^5, 7.94×10^5, 9.05×10^5 , 8.94×10^5 and 7.68×10^5(cfu/gm), respectively. All types of cheeses revealed non significant ($P>0.05$) differences as shown in Table 1. However, with the exception of cheese made from sheep's milk there were significant ($P<0.05$) differences in coliform count for all types of cheese during storage period (Table 3).

Yeast and mould count

The means of yeast and mould count for cheeses made using camel's milk, camel's milk: sheep's milk (75%: 25%), camel's milk: sheep's milk (50%:50%), camel's milk: sheep's milk (25%: 75%) and sheep's milk were 5.44×10^5, 7.05×10^5, 7.71×10^5, 5.58×10^5 and 6.57×10^5 (cfu/gm), respectively. ***Non*** significant ($P>0.05$) differences were found in the values of yeast and mould counts for different types of cheeses as shown in Table 1. There were significant ($P<0.05$) differences of the yeast and mould counts for all types of cheese during storage period except for that made using sheep's milk (Table 4).

Psychrotrophic count

Values of psychotrophic bacterial count for cheeses made using camel's milk, camel's milk: sheep's milk (75%: 25%), camel's milk: sheep's milk (50%:50%), camel's milk: sheep's milk (25%: 75%) and sheep's milk were 8.69×10^5 , 9.43×10^5 , 8.85×10^5, 8.94×10^5 and 9.07×10^5 (cfu/gm), respectively, as shown in Table 1. Non significant ($P>0.05$) differences were obtained for cheeses due to variations of types of milk and storage period (Table 5).

4. DISCUSSION

The high total bacterial counts in camel milk were in accord with the findings of Semereab and Molla (2003); Khedid et al. (2003); Sheuip et al. (2007) and Mohamed and El Zubeir (2012). This might be due to either insufficient heating of milk and/or the lower initial survival rate of bacteria in cheese, which increased during cheese storage. The mean of total bacterial count for camel's milk cheese was log 9.44 compared with log 9.20 reported by Ahmed and El Zubeir (2011). The TBC of camel milk was reported with values that vary between 10^2 and 10^8 cfu/ml (Semereab and Molla, 2001; Wernery et al., 2002; Sela et al., 2003 and Sheiup et al., 2007).

Table 4: Effect of the storage period on yeast and mould count of cheeses made from camel's, sheep's milk and their mixtures

Types of cheeses	Storage period (days)					L.S
	1	3	6	15	21	
	Means± SD	Means± SD	Means± SD	Means± SD	Means± SD	
Camel's milk	$4.02\times10^5\pm 50.36^{bc}$	$7.46\times10^5\pm.4\ 7.64^{a}$	$6.89\times10^5\pm 48.63^{ab}$	$3.21\times10^5\pm 27.66^{bc}$	$1.92\times10^5\pm16.43^{c}$	*
Camel's milk: sheep's milk (75%: 25%)	$6.92\times10^5\pm 57.29^{ab}$	$8.83\times10^5\pm 60.05^{a}$	$6.54\times10^5\pm 45.90^{b}$	$5.10\times10^5\pm 25.16^{b}$	$3.86\times10^5\pm 34.89^{b}$	*
Camel's milk: sheep's milk (50%:50%)	$5.20\times10^5\pm 47.36^{b}$	$9.27\times10^5\pm 46.87^{a}$	$8.36\times10^5\pm 56.21^{ab}$	$6.97\times10^5\pm 44.15^{b}$	$3.98\times10^5\pm 47.58^{b}$	*
Camel's milk: sheep's milk (25%: 75%)	$5.34\times10^5\pm47.3\ 3^{abc}$	$11.33\times10^5\pm67.34^{a}$	$7.61\times10^5\pm 47.51^{ab}$	$3.29\times10^5\pm 26.04^{bc}$	$3.40\times10^5\pm 10.46^{c}$	*
Sheep's milk	$7.92\times10^5\pm 53.16^{a}$	$12.27\times10^5\pm53.31^{a}$	$8.11\times10^5\pm 61.58^{a}$	$5.91\times10^5\pm 42.43^{a}$	$4.91\times10^5\pm 52.60^{a}$	N.S

a, b, c :Values in the same row bearing different superscript letters are significantly different ($P < 0.05$)
L.S = Significant level N.S = Not significant * = ($P<0.05$) SD = Standard deviation

Table 5: Effect of the storage period on psychrotrophic count of cheese made from camel's, sheep's and their mixtures milk

Types of cheeses	Storage period (days)					L.S
	1	3	6	15	21	
	Mean± SD	Mean± SD	Mean± SD	Mean± SD	Mean± SD	
Camel's milk	$7.03\times10^5\pm 68.88^{a}$	$10.99\times10^5\pm 111.90^{a}$	$5.82\times10^5\pm75.51^{a}$	$7.27\times10^5\pm 63.62^{a}$	$4.36\times10^5\pm 78.66^{a}$	N.S
Camel's milk: sheep's milk (75%: 25%)	$12.05\times10^5\pm86.49^{a}$	$12.29\times10^5\pm 89.13^{a}$	$7.03\times10^5\pm 52.12^{a}$	$9.33\times10^5\pm67.51^{a}$	$6.46\times10^5\pm 51.35^{a}$	N.S
Camel's milk: sheep's milk (50%:50%)	$9.17\times10^5\pm 60.25^{a}$	$10.94\times10^5\pm74.14^{a}$	$6.90\times10^5\pm 51.05^{a}$	$11.98\times10^5\pm 79.72^{a}$	$6.71\times10^5\pm 61.88^{a}$	N.S
Camel's milk: sheep's milk (25%: 75%)	$10.57\times10^5\pm94.96^{a}$	$11.56\times10^5\pm 69.13^{a}$	$8.38\times10^5\pm42.76^{a}$	10.99 ×105± 85.75a	$5.22\times10^5\pm 19.38^{a}$	N.S
Sheep's milk	$8.92\times10^5\pm 73.98^{a}$	$11.31\times10^5\pm 69.92^{a}$	$9.90\times10^5\pm 42.83^{a}$	$11.92\times10^5\pm 82.93^{a}$	$8.31\times10^5\pm 83.18^{a}$	N.S

:Values in the same row bearing different superscript letters are significantly different ($P < 0.05$)
L.S = Significant level N.S = Not significant SD = Standard deviation

These differences underline the fact that TBC depends on several factors including the camel milk itself, contamination of the camel udder and contamination of milking personnel, containers and milking conditions of the camels (Younan, 2004).

Means of total bacterial count for all five types of cheeses were high during the first day and then gradually decreased in day 21 (Table 2). The high TBC was possibly attributed to the growth of microorganisms during early stages of ripening, which might be explained by the microbial counts of raw milk. With the exception of camel milk cheese significant ($P<0.05$) differences were obtained during the storage period in the total bacterial count of camel's milk cheese; similar results were reported by Ahmed and El Zubeir (2011). However the decrease obtained in the TBC is likely due to the effect of high lactic acid in the cheese samples (Shakeel-Ur Rehman et al., 2000).

Means of coliform count for all five types of cheese except sheep's milk cheese were high during the first day and then gradually decreased at day 21 (Table 1 and 3). This might be due to the lack of proper handling and hence contamination by microorganisms as most of camel owners practiced less hygiene during milking and storage of their milk (Shuiep et al., 2007). The coliform count of camel's cheese decreased from log 9.08±77.73 at the first day to log 2.74 ±8.73 on the 21th day, this may be due to the fact that camel milk was reported to have an antimicrobial effect against Gram positive and Gram negative bacteria, including *Escherichia coli*, *Listeria monocytogenes, Staphylococcus aureus* and *Salmonella typhimurium* (Benkerroum et al., 2004). The inhibitory action of camel milk against *L. monocytogenes*, *S. aureus* and *E. coli* might be attributed to the presence of lactoperoxidase, hydrogen peroxide and lysozyme (Benkerroum et al., 2004). The growth of *S. typhimurium* was inhibited by lactoferrin in camel milk through binding iron and making it unavailable for its growth (Ochoa and Cleary, 2009). Significant (P<0.05) differences were obtained during the storage period in coliform count of camel's milk cheese, these results are in line with those found by Ahmed and El Zubeir (2011). Saad et al. (2013) recommended good hygienic practices in milk production to achieve good microbiological quality of sheep milk.

Tables 1 and 4 showed that the impact of the type of milk and mixing on the microbiological properties of cheeses did not revealed significant (P>0.05) differences in the count of yeasts and mould with different types of cheeses. Significant differences were obtained in the count of yeasts and moulds during the storage period of each type of cheese as they revealed a continuous decrease with progress in storage time. Significant (P<0.05) differences were obtained during the storage period in the yeasts and mould count of camel's milk cheese, these results are in line with those found by Ahmed and El Zubeir (2011). The yeasts and mould count of camel's cheese decreased from log 4.02± 50.36 at the first day to log 1.92± 16.43 on the 21th day, the overall mean of yeasts and mould count of camel's cheese was log 5.44± 46.71 compared with log 9.28± 0.237, which was reported by Ahmed and El Zubeir (2011). Yeasts are not added as part of starter culture during cheese manufacture. However, relatively high counts were frequently observed in many soft, semi-soft and surface ripened cheeses, probably originating from the processing equipment and the dairy environment (Viljoen, 2001).

The psychrotrophic bacterial count values for camel's milk cheese had no significant (P>0.05) differences during storage period (Tables 1 and 5). Similar results were reported by Ahmed and El Zubeir (2011) when preparing cheese from camel milk by using different salt levels. The psychrotrophic count of camel milk cheese present in this study was log 8.69± 73.50 compared with that reported by Ahmed and El Zubeir (2011) who found log 7.2± 0.458. The psychrotrophic count values for sheep's milk cheese observed in this study was log 9.07± 73.23 compared with that reported by Santos et al. (1996). They found log 3.90± 1.01 and log 6.94± 0.65 for Villalon cheese made from pasteurized sheep milk and raw sheep milk, respectively. Because psychorotropic bacteria produce heat resistant lipases and proteases which may reduce yield of cheese and produce undesirable flavors in the ripened cheese (Skeie, 2007), proper heating of milk should be considered. Psychrotrophs are the bacteria that normally limit the shelf life and decrease the yield and quality of cheese curd (Fairbairn and Law, 1986; Mohamed and Bassette, 1979 and Nelson and Marshall, 1979). Moreover it was reported that proteases produced by some psychrotrophes stimulate plasminogen activators, transformed plasminogen to plasmin (Frohbieteter et al., 2005).

Te non Significant (P>0.05) differences obtained during the storage period in psychrotrophic count for the cheeses made of mixtures of camels and sheep's milk might indicated psychrotrophic bacteria present in the cheeses are originating from the raw milk.

5. CONCLUSIONS

The present study concluded that there were significant ($P<0.05$) decrease in the total bacterial count, yeast and mould count and coliform count for all types of cheeses during storage period excepted sheep's milk cheese. However there were non significant ($P>0.05$) differences in the values of psychotrophic bacterial count for all five types of cheeses during storage period.

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