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Microbiology of Camel Fermented Milk (Gariss) in Sudan*

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Abstract: The present study is a trial for processing gariss (Sudanese camel fermented milk) in the laboratory and to investigate the effect of pasteurization and storage conditions on its microbial content. Gariss that was used as a culture for fermentation showed total bacterial count of 1.8×10^6, *Lactobacillus* spp. count of 4.9×10^6 and yeast count of 4.9×10^6. Pasteurization and storage periods revealed significant variations ($p<0.05$) for total bacterial count. Similarly, *Lactobacillus* spp. count was found to show significant ($p<0.05$) variations by the incubation temperatures. Also the storage conditions (temperature and duration) revealed significant differences ($p<0.05$) between the processed samples for yeast count. The processed samples were found to show shelf life of up to 10 days. The present study concluded that the microbial contents were affected by pasteurization and storage conditions (temperatures and storage periods). Hence the study recommended that more investigations are needed on the microbiological properties of camel's milk products.

Key words: Microbiological properties, fermented camel's milk, gariss, Sudan

INTRODUCTION

Numerous microorganisms, including bacteria, yeasts and molds, constitute the complex ecosystem present in milk and fermented dairy products (Ogier *et al*., 2004). The microorganisms that responsible about lactic acid and alcoholic fermentation are lactic acid bacteria (Attia *et al*., 2001). These microorganisms are commonly found in a symbiotic relationship in many fermented milks of the world (Dirar, 1993).

Lactic acid bacteria (LAB), especially the genus Lactobacillus, followed by Enterococcus, Lactococcus and Leuconostoc, dominated the microflora of kule naoto, the traditional fermented milk products of the Maasai in Kenya (Mathara *et al*., 2004). They also added that the major *Lactobacillus* species was *Lactobacillus plantarum* (60%) and a lower frequency of isolation for *Lactobacillus fermentum, Lactobacillus paracasei* and *Lactobacillus acidophilus.*

The microflora involved in production of suusac, a Kenyan traditional fermented camel milk product, were enumerated and identified and a total of 45 LAB and 30 yeast isolates were isolated from the 15 suusac samples. 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* (Lore *et al*., 2005). Similarly Shuangquan *et al.* (2004) studied the microflora in traditional fermented camel's milk, Hogormag which is made by nomadic families in the Inner Mongolia Autonomous Region. They identified the isolated lactic acid bacteria as

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Enterococcus faecium, *Lactococcus lactis subsp. cremoris*, *Leuconostoc lactis*, *Lactobacillus acidophilus*, *Lb. helveticus* and *Lb. plantarum* and *Lb. bavaricus*. The yeasts that were isolated were identified as *Candida kefyr*, *Saccharomyces cerevisiae*, *C. krusei* and *C. glabrata*. However, Robinson (1981) indicated that in the dairy industry *Lact. bulgaricus*, *Lact. lactis*, *Lact. acidophilus*, *Lact. helveticus*, *Lact. caseii* and *Lact. plantarum* are most commonly used organisms. Similarly Gobbetti *et al*. (2000) mentioned that *Lactobacillus delbrueckii subsp. bulgaricus* and *Lact. lactis subsp. cremoris* are two of the most widely used industrial strains which are used as starters for fermented milks and several types of cheeses. Moreover, Robinson (1981) mentioned that *Str. thermophilus* is used symbiotically with *Lactobacillus bulgaricus* as a yoghurt and cheese starter culture.

Yagil (1982) indicated that fermented products of camel's milk have various names in various parts of the world. In the Sudan gariss is special kind of fermented milk, prepared solely from camel's milk under more or less continuous shaking (Dirar, 1993).

Gariss fermentation is attributed to lactic acid bacteria and alcohol producing yeasts (Mirghani, 1994). Gariss thus falls in the family of acido-alcoholic fermented milks that include kefir and koumis (Dirar, 1993). He also indicated that the direct microscopic observations of gariss samples obtained from Butana area and Northern Kordufan Province revealed two types of microorganisms, which are lactobacilli and yeast. Also a limited numbers of streptococci are found as he reported.

The aim of the present study is processing of gariss in the laboratory after heat treatment using a ready starter gariss, in order to assess the microbial content of the processed samples and to assess its shelf life.

MATERIALS AND METHODS

Sources of Samples

The present study was conducted during the period from January to April 2004. Fresh milk for preparation was obtained from Khartoum North (Elhag Yosif area). Half of the quantity of the camel milk (1250 mL) was pasteurized at 63°C for 30 min, as mentioned by Attia *et al*. (2001). Acidification of the raw fresh and pasteurized milk was carried out by the addition of 50 mL fractions of a ready gariss that obtained from Elhag Yosif area as a culture. After inoculation each of the sample was divided into 625 mL fractions, 312.5 mL fractions (pasteurized and non pasteurized gariss samples) were incubated at the room temperature about 25°C and the other was incubated at 37°C.

Analysis of the Samples

The analysis was done at the laboratory of the Department of Dairy Production, Faculty of Animal Production of University of Khartoum. The prepared gariss samples were examined for total bacterial counts, *Lactobacillus* spp. counts, *Streptococcus* spp. counts and yeast counts. The microbial counts were carried out after one hour from inoculation, 3, 18 and 42 h. The counts were then done periodically each 48 h for 10 days.

Sterilization, serial dilution and preparation of the media were done according to Harrigan and MacCance (1976). Standard plate count agar and Malt extract agar were used for total bacterial count and yeast counts, respectively (Harrigan and MacCance, 1976). *Lactobacillus* spp. count was determined on MRS agar and the count of *Streptococcus* spp. was determined on M-17 agar (Attia *et al*., 2001). Isolation and identification of the purified colonies was carried out according to Harrigan and MacCance (1976) and Barrow and Felthman (1993).

Statistical Analysis

Experiment was conducted using Completely Randomize Design. The analysis of variance and the significant differences between means were determined using Duncan Multiple Range test using SPSS.

RESULTS

Microbial Content of Processed Fermented Camel Milk Samples

The acidity and pH of the gariss culture was found to be 2.49% and 3.8, respectively. The total bacterial count, *Lactobacillus* spp. count and yeast count of the gariss culture were found to be 1.8×10^6, 4.9×10^6 and 4.9×10^6, respectively. However, the fresh camel milk, which collected for the processing was found to have acidity and pH of 0.12% and 6.4, respectively.

Microbial Content of the Processed Gariss Samples

Log Total Bacterial Counts

Table 1 showed that after one hour incubation the mean log total bacterial count revealed values of 4.1±0.001, 4.41±0.006, 4.36±0.011 and 4.54±0.027, respectively. At 186 and 234 h the reduction of means log total bacterial counts of the samples were recorded. However, at the end of the storage period (234 h) the mean log revealed values of 6.24±0.011, 6.69±0.012, 6.48±0.02 and 6.76±0.003, respectively.

Table 1 also showed significant differences ($p<0.05$) between gariss samples made from pasteurized milk and that made from non pasteurized milk. However, it revealed significant variations ($p<0.05$) between the samples during the different storage periods.

Log *Lactobacillus* spp. counts

The mean *Lactobacilli* spp. counts in gariss samples made from pasteurized milk that incubated at both 25 and 37°C and gariss samples made from non pasteurized milk that incubated at 25 and 37°C after 1 h incubation were found to be 3.24±0.088, 3.68±0.324, 3.58±0.032 and 3.71±0.012, respectively (Table 2). The same table also showed gradual increase in the Log *Lactobacillus* spp. counts in 5 h of the storage period. After 42 h incubation, gariss samples were found to be 5.1±0.011, 5.76±0.018, 5.44±0.033 and 5.53±0.029, respectively. The average reduction in the log *Lactobacillus* spp. was recorded at 186 and 234 h of incubation. The mean log *Lactobacillus* spp. counts at 234 h incubation were found to be 5.45±0.081, 5.99±0.000, 5.21±0.63 and 6.28±0.014, respectively.

Table 1: Comparison of log total bacterial counts of the processed gariss samples made from pasteurized and non pasteurized camel milk incubated at different temperatures for 234 h

Storage periods (h)	Past. 25°C	Past. 37°C	Non past. 25°C	Non past. 37°C	SL.of means
1	4.10±0.001	4.411±0.006	4.36±0.011	5.54±0.021	4.33^a
3	4.46±0.021	4.660±0.003	4.57±0.005	4.78±0.003	4.62^b
5	4.60±0.004	4.820±0.002	4.67±0.009	4.88±0.003	6.74^c
18	4.72±0.026	4.910±0.006	4.70±0.000	4.93±0.003	4.82^d
42	5.91±0.000	6.300±0.009307	6.16±0.013	6.45±0.011	6.20^e
90	6.71±0.001	6.890±0.006	6.85±0.001	6.90±0.004	6.84^h
138	6.81±0.008	6.910±0.006	6.87±0.006	6.93±0.002	6.88^i
186	6.5054±0.00911	6.830±0.009	6.79±0.005	6.88±0.005	6.75^g
234	6.24±0.011	6.691±0.012	6.48±0.02	6.76±0.003	6.5424^f
SL. of means	5.55^a	5.82^c	5.72^b	5.89^d	

In this and the following tables: Different superscript letter(s) on the same column and raw indicated significant differences at $p\leq0.05$

Table 2: Comparisons of log lactobacilli counts of processed gariss samples made from pasteurized and non pasteurized camel milk incubated at different temperatures for 234 h

Storage periods (h)	Past. 25°C	Past. 37°C	Non past. 25°C	Non past. 37°C	SL of means.
1	3.24±0.088	3.6761±0.03236	3.5792±0.03236	3.7075±0.01204	3.5503[a]
3	3.72±0.040	4.0952±0.002467	3.9022±0.03844	4.2527±0.01373	3.9944[b]
5	4.10±0.004	4.30±0.008	3.950±0.007	4.36±0.009	4.2[c]
18	4.3±0.015	4.41±0.012	4.337±0.01	4.50±0.000	4.39[d]
42	5.10±0.02	5.76±0.018	5.440±0.033	5.53±0.029	5.46[e]
90	6.30±0.009	9.51±0.003	6.420±0.001	5.55±0.009	6.19[g]
138	6.39±0.01	6.55±0.006	6.480±0.007	6.52±0.096	6.48[h]
186	5.86±0.003	6.10±0.000	6.150±0.021	6.32±0.038	6.11[g]
234	5.46±0.081	5.99±0.000	5.210±0.63	6.28±0.011	5.23[f]
SL. of means	4.93[a]	5.26[c]	5.05[b]	5.22[c]	

Table 3: Comparison of log yeast counts of the processed gariss samples made from pasteurized and non pasteurized camel milk incubated at different temperatures for 234 h

Storage periods (h)	Past. 25°C	Past. 37°C	Non past. 25°C	Non past. 37°C	SL. of means
1	3.48±0.000	3.83±0.002	3.67±0.039	4.23±0.047	3.81[a]
3	3.80±0.025	4.30±0.003	4.30±0.02	4.30±0.003	4.1[b]
5	4.23±0.11	4.47±0.006	4.30±0.008	4.60±0.005	4.4[c]
18	4.44±0.007	4.58±0.008	4.49±0.015	4.70±0.009	4.55[d]
42	5.37±0.011	5.89±0.012	5.74±0.045	5.89±0.018	5.72[e]
90	6.44±0.032	6.60±0.005	5.55±0.006	6.62±0.732	6.55[h]
138	6.55±0.007	6.63±0.012	6.53±0.003	6.69±0.04	6.6[i]
186	6.13±0.02	6.42±0.000	6.35±0.004419	6.59±0.02	6.37[g]
234	5.88±0.092	6.26±0.001	6.037±0.02678	6.43±0.004	6.15[e]
SL. of means	5.14[a]	5.44[c]	5.3001[b]	5.57[d]	

Moreover, the higher increase was observed in the mean log *Lactobacillus* spp. count of gariss samples made from non pasteurized milk that incubated at 37°C. However the lower rate was recorded for gariss samples made from pasteurized milk which incubated at 25°C. Table 2 also revealed significant variations ($p<0.05$) between gariss samples made from pasteurized and non pasteurized milk which incubated at 25°C. Moreover, gariss samples made from non pasteurized milk that incubated at 37°C revealed significant differences ($p<0.05$). However, non significant variation ($p>0.05$) were found when comparing gariss samples made from pasteurized and non pasteurized milk which incubated at 37°C.

Log Yeast Counts

Table 3 showed variations in the increasing rate of the means log yeast counts. Gariss samples made from pasteurized milk that incubated at 25°C revealed the lower mean log counts. However, higher mean values were recorded for gariss samples that made from milk, which incubated at 37°C. The data also revealed that the means log yeast counts for gariss samples were 3.48, 3.828±0.002, 3.67±0.039 and 4.23±0.047, respectively. Moreover, the means log yeast count after 42 h of storage reached values of 5.36±0.011, 5.8866±0.01204, 5.7416±0.05421 and 5.8863±0.01765, respectively. At the end of the storage period (234 h) those samples revealed means log yeast counts of 5.8781±0.09216, 6.2607±0.0008425, 6.037±0.02678 and 6.432±0.003688, respectively. Table 3 also showed significant variations ($p<0.05$) due to pasteurization and storage conditions.

DISCUSSION

The microbial content of processed gariss revealed that the total bacteria count showed minimum rate of growth at the beginning of the incubation. This supported Attia *et al*. (2001) who reported that the activity of the starter in dromedary milk was characterized by a longer lag phase

(approximately 5 vs. approximately 1 h) and by an earlier decline phase than bovine milk. This might occur due to the presence of growth inhibiting– factors in the camel's milk (Gran, 1991; Attia *et al*., 2001). The present results also showed that both pasteurization and incubation temperature affected the total bacteria counts. Since the lower mean values were recorded for gariss samples made from pasteurized milk that incubated at 25°C (Table 1). These results were in agreement with the IDF (1994) and Harding (1999). Also IDF (1994) stated that in the manufacture of milk based products, a heat treatment process may perform a technological function, for example to increase heat stability and prevent syneresis. The procedure of heat treatment if introduced to camel owners who process those traditional products, it would improve their product by killing the pathogenic microorganisms and increasing the shelf life (Harding, 1999) and hence safe product could be commercialized.

The *Lactobacillus* spp. counts revealed the same pattern of the total bacteria count at the beginning of the storage periods, since they revealed along lag phase (Table 2). This was in accord to the previous findings of Attia *et al*. (2001) that the activity of the starter in dromedary milk was characterized by a longer lag phase. However yeast counts (Table 3) showed high rate of increase at the beginning of the incubation than the *Lactobacilli* spp. counts. The source of yeast in Gariss prepared during the present study was the starter Gariss as in the present study we use the traditional starter from the nomadic owners who supplied the camel milk. Yeasts are present in indigenous African fermented milks in numbers up to log 8 cfu g^{-1}, together with a varied Lactic Acid Bacteria (LAB) flora and therefore potentially contribute to product characteristics (Narvhus and Gadaga, 2003).

The processed gariss samples were found to have a high keeping quality, since the shelf life of the product was extended up to 234 h at both 25 and 37°C. The processed gariss samples revealed longer shelf life than yoghurt which revealed a shelf life of 10 days when kept under refrigeration (Robinson, 1981). The variations in the shelf life between the two products might occur due to the presence of antibacterial and antimicrobial agents in the camels' milk (Elagamy, 1992). Moreover, pasteurization of the milk had an effect on the keeping qnality of the product, since the heat treatment is used to kill the pathogens and to extend the storage life of the products (Harding, 1999). The significant rate of increase for the total bacterial counts, *Lactobacillus* spp. counts and yeast counts and their significant decrease at the end of storage periods indicated a prolonged keeping quality and that minimum spoilage organisms are present. The spoilage of the product was found to occur after 234 h of incubation period in the two storage temperatures (25 and 37°C). The spoilage of the non pasteurized fermented camel's milk might occur due to the presence of lower initial numbers of contaminated yeast.

The present study concluded that pasteurization and fermentation improve the quality of camel milk, since gariss revealed longer shelf life in the different storage conditions which used. The high temperature (25 and 37°C) used during this study might be more or less similar to the storage condition of the traditional gariss (kept in a leather bags at the back of one of the camel). Hence it is recommended that heating of milk prior to its processing into any dairy products should be introduced to camel herders. Also establishment of mobile dairy factory for processing of pasteurized camel milk and fermented products is urgently needed. Further research is needed to address the fermentation process of camel milk with special references to the microbial content and its properties. Isolation, identification and molecular characterization of fermentative microorganisms, which found in the camels milk products comparing with the other microorganisms present in other animal milk products is urgently needed.

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