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Hygienic Quality of Camel Milk and Fermented Camel Milk (*Chal*) in Golestan Province, Iran

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Abstract A total of 18 samples of fresh camel milk and sour camel milk (chal) were randomly collected from different retail markets with various levels of sanitation in Golestan province. Samples were subjected to determinations of pH, total count, total yeast and mould counts, Enterobacteriaceae count, lactic acid bacteria (LAB) count, total Staphylococcal count and for presence of *Salmonella* and *Shigella*. Results revealed that pH levels ranged from 3.8 to 4.5 and 6.4 to 6.7 for chal and milk samples respectively. The highest and lowest total counts were determined in the ranges of 5.90 to 5.69, and 5.69 to 5.46; total yeast and mold counts were determined in the ranges of 4.11 to 3.90 and 3.77 to 1.95, and *Enterobacteriaceae* counts were 2.60 to 1.90 and 2.04 to 1.77, *Lactic acid bacteria* counts were 4.58 to 4.04 and 2.60 to 1.95, *Staphylococcal* counts were 1.95 to 1.30 and 2.00 to 1.00 log cfu/ml for chal and camel milk samples respectively. *Salmonella* and *Shigella* were not found in any of the chal samples, but there were traces in 8 of the raw camel milk samples. But it should be considered that chal is prepared under spontaneous fermentation, and in comparisson with pasteurized products it has low sanitation quality.

Keywords Chal, pH, *Salmonella*, Sanitation Quality, Enterobacteriaceae

1. Introduction

Camel milk and chal (soured camel milk) are consumed by some people in Iran and in other countries in Asia and Africa. Chal is prepared by adding water to raw camel milk and spontaneous fermentation takes place in a skin bag or a bottle at ambient temperature [23]. Camel milk and fermented camel milk have nutritional and medical properties that make them valuable foods. Chal contains some useful lactic acid bacteria [26-28]. In many regions, camel milk and chal are used to treat some diseases and to combat health problems such as dropsy, jaundice, tuberculosis, asthma, anaemia and piles [35]. Tests showed that patients with chronic hepatitis improved after consumption of camel milk [41]. Foods that promote good health and prevent disease are valued highly by today's consumers. It should be considered that chal is made from raw milk by spontaneous fermentation, so it may contain some harmful organisms.

Dairy products generally constitute a favourable environment for the growth of yeasts, lactic acid bacteria and other acid tolerant microorganisms because they have an acidic pH and contain nutritious compounds [51-37]. Yeasts usually coexist with lactic acid bacteria in dairy products. One of the groups will gain dominance over the other, or both groups grow together and specific interactions take place.

The aim of the present work was to assay microbial quality of camel milk and chal in Golestan province, Iran

2. Materials and Method

Samples of camel milk and chal were randomly collected from different households and retail markets with various levels of sanitation in Golestan province. The areas under investigation were the cities of Gonbad, Aghghala and Bandar Torkman. Samples were transported to the labratoary of the department of food science and technology, University of Gorgan and subjected to determination of chemical and microbial characteristics.

2.1. Compositional Analysis

Composition properties of samples were analyzed in duplicate for contents of protein, fat, ash, pH, TS, NaCl and acidity. The Kjeldahl method was used to determine total protein content of camel milk and chal, TS using a drying oven and Titrable acidity was determined by titration with 0.1 N NaOH using phenolphthalein as a color indicator until the color changed to light pink and persisted for 30 seconds, volume of NaOH was recorded and results were expressed in degrees (Dornic) [7]. Fat content was measured by the

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Gerber method [36] and ash by heating samples in a muffle furnace at 100°C for 1 hour, 200°C for 2 hours and 550°C overnight [30]. Levels of pH were determined using a pH meter (766knick, Germany) and NaCl evaluations were measured according tothe method cited in Bradley et al. [10].

2.2. Microbiological Assay

Microbiological characteristics of camel milk and chal such as total count, total yeast and mould count, Entrobacteriaceae, staphylococcal count and *Shigella* were tested according to Standard Methods for the Examination of Dairy Products [17]. S*almonella* count was determined according to the method cited in Andrew and Jacobson [6], and lactic acid bacteria count was determined according to the method described in the Compendium of Methods for the Microbiological Examination of food [43]. For S*almonella* count, each 25g sample was aseptically weighed and macerated and 225 mls of sterile distilled water was added. Sterile dilution was carried out using sterile distilled water as the dilutant from each dilution, 1 ml was plated using the pour plate method cited in Andrew and Jacobson [6].

For total count, total yeast and mould, Entrobacteriaceae, staphylococcal count, *Shigella* and lactic acid bacteria count, samples were diluted in sterile distilled water (to get readily countable numbers of microorganisms) and plated in Plate Count Agar (Mirmedia, Mp-2602, Iran) for total counts, in Baird Parker agar (Micro media, Mm0213, Hungary) for *Staphylococcus aureus* count, in VRBA (Micro media, Mm0114, Hungary) for Entrobacteriaceae counts, in MRS agar (Liofilchem, 610024, Italy) for lactic acid bacteria and in YGC agar (Mirmedia, Mfd, Iran) for yeast and mould counts. Plates were incubated at 37°C for 48 h for bacteria and at 30°C for 5 d for yeast and mould counts.

3. Results and Discussion

3.1. Composition Analyses

Chemical properties of camel milk and chal were determined and results are shown in Tables 1 and 2.

Results (Tables 1 and 2) revealed that pH ranged from 3.8 to 4.5 and 6.4 to 6.7 and acidity levels were 29.8 to 36 and 15 to 17.3 for chal and milk respectively. However higher values for pH and acidity were also recorded [31-29]; but the average pH recorded for camel milk was between 6.2-6.8 [22]. It can increase to 7.2 in cases of clinical mastitis [45]. Lower pH is considered inhibitory to vegetative cell growth of pathogenic microorganisms, but helpful to yeast growth, molds and lactic acid bacteria.However similar results were found in the related literature [3] but fermented foods are normally considered as safe against food-born diseases because of their low pH [21].

3.2. Microbiological Quality

Microbial count of Chal and camel milk are shown in Tables 3 and 4. Results for chal samples showed significance in terms of yeast and mold counts (Table 3). Yeast and mould counts ranged from 4.11 to 3.9 and 3.77 to 1.95 log cfu/ml for chal and camel milk samples, respectively.

Table 1. Chemical Analysis of Chal Samples

Sample NO.	Protein%	Fat%	Total Solids %	Ash%	Acidity (Dornic)	pH	NaCl%	Alcohol% (w/v)
1	1.3	2	4.1	0.32	32	4	0.41	0.4
2	1.4	2.1	4.3	0.3	33	4.2	0.35	0.5
3	1.2	2.2	4.2	0.35	29.8	4.5	0.3	0.5
4	1.4	2.3	4.5	0.4	30	4.4	0.34	0.6
5	1.5	2.5	4.6	0.44	35	4.5	0.32	0.7
6	2	2.3	5	0.45	34	4	0.4	0.6
7	1.6	2.1	4.4	0.42	35	3.9	0.33	0.5
8	1.8	1.6	4.1	0.38	36	3.8	0.32	0.5
9	1.4	1.9	4.2	0.37	32	4.5	0.38	0.6

Table 2. Chemical Analysis of Camel Milk Samples

Sample NO.	Protein%	Fat%	Total Solids%	Ash%	Acidity (Dornic)	pH	NaCl%	Alcohol% (w/v)
1	3.2	3	12	0.9	15	6.7	0.8	0
2	3.3	3.2	12.5	0.85	16	6.6	0.75	0
3	3.1	3.4	12.4	0.92	15.5	6.6	0.8	0
4	3	3.2	12.3	0.98	16	6.7	0.85	0
5	3.5	3.1	12.4	0.8	17	6.5	0.7	0
6	3.4	3.1	12.2	0.87	16.5	6.5	0.8	0
7	3.3	3.2	12.2	0.85	17.3	6.4	0.76	0
8	3.6	3	12.1	0.8	16.2	6.6	0.7	0
9	3.2	3.2	12.2	0.86	15.8	6.7	0.8	0

Table 3. Microbial Count of Chal Samples (log cfu/ml)

Sample NO.	1	2	3	4	5	6	7	8	9
Total count	5.90	5.82	5.85	5.83	5.79	5.76	5.69	5.75	5.71
Yeast mould	4.11	4.04	3.90	4.04	3.95	4.00	4.08	4.05	4.02
Lactic acid bacteria	4.58	4.51	4.44	4.14	4.04	4.46	4.32	4.08	4.25
Enterobacteriaceae	2.60	2.30	2.30	2.00	1.90	1.95	2.30	2.47	1.95
Staphylococ. count	1.95	1.78	1.78	1.69	1.84	1.69	1.69	1.47	1.30
Salmonella	< 0	< 0	< 0	< 0	< 0	< 0	< 0	< 0	< 0
Shigella	< 0	< 0	< 0	< 0	< 0	< 0	< 0	< 0	< 0

Table 4. Microbial Count of Camel Milk Samples (log cfu/ml)

Sample NO.	1	2	3	4	5	6	7	8	9
Total count	5.69	5.61	5.60	5.58	5.50	5.56	5.46	5.55	5.49
Yeast moulds	3.77	3.60	2.90	2.84	2.47	2.77	1.95	2.85	2.77
Lactic acid bacteria	2.60	2.56	2.57	2.38	2.00	2.30	1.95	2.25	2.17
Enterobacteriaceae	2.04	2.00	1.90	1.77	1.77	1.95	1.90	1.77	1.84
Staphylococcal. count	2.00	1.60	1.47	1.30	2.00	1.65	2.00	1.47	1.00
Salmonella	+	+	< 0	+	< 0	+	< 0	< 0	< 0
Shigella	< 0	< 0	+	+	< 0	< 0	< 0	+	+

Results revealed that yeasts, moulds and lactic acid bacteria counts in chal were higher in comparisson to those of camel milk. This can be attributed to the fermentation process and acidic pH of the chal samples. Benkerroum et al. [9] reported high counts (4.6 log cfu/ml) of yeasts and moulds in camel milk tests in Morroco that were higher than those of our results, also Abdel Gadir et al. [2] found a high count of yeasts in cow milk. In this study yeasts and moulds existed in all examined samples. El-Ziney and Al-Turki [17] identified yeasts and moulds in 57% of the camel milk samples with mean and maximum values of 1.9 and 5.65 log cfu/ml, respectively.

Frazier and Westhoff [20] and Almaw and Molla [34] reported that high counts of yeasts and moulds in milk are uncommon because of the natural pH level of milk that causes bacteria to predominate. El-Jakee [16] found *Candida albicans* in 4% of camels with clinical mastitis. Presence of yeasts might be atributed to contamination by air, water or a lack of proper hygenic practice during the production process, while the presence of moulds was usually attributed to contamination of the product by air or persons who were engaged in its preparation or transportation.

Total count recorded for chal was higher than that of camel milk and this may be attributed to growth and high yeast content of moulds and LAB (lactic acid bacteria) in Chal from the fermentation process.

In this study, the mean total counts recorded 5.56 and 5.78 log cfu/ml for camel milk and Chal respectively. The results of Wernery et al. [50] proved the total count values of camel milk (hand milking) were of 10^2 - 10^4 Cfu/ml, but they were lower than values given by Younan [52] in which total bacteria count was reported as10^3 - 10^5 Cfu/ml.

The mean of the total aerobic mesophilic bacteria count in the Qasim region reported 5 log cfu/ml with a maximum 7.15 log cfu/ml. [18] and for Saudi Arabia 5.4 log cfu/ml in average by Al Mohizea [5] and 5.6 log cfu/ml for a study on camel milk in Ethiopia by Semereab and Molla [39] and about 6 logcfu/ ml by Elgadi et al. [15].

The total bacteria count (TBC) of camel milk was reported to vary between 10^2 and 10^8 cfu/ml [39-50-38-52]. These differences show that TBC depends on several parameters such as the milk itself, contamination of the camel's udder, milking personnel and other considerations such as transportation and containers. The results for total count in this study were in aggreement with reports from Saudi Arabia, Ethiopia and Kenya fortests on camel milk samples by Al Mohizea [5], Semereab and Molla [39] and Younan [52] respectively.

Under pastoral production conditions, environmental contamination plays a bigger role in hygiene levels of raw camel milk and fermented camel milk products than initial bacterial contamination of the camel's milk itself [52]. If total bacterial count is determined as low, it was observed that raw milk did not to turn sour for up to 4 days when it was kept in a clean container and refrigerated [52].

Staphylococci were present in all examined samples. Highest and lowest counts recorded for chal and camel milk samples were 1.95, 1.30 and 2.00, 1.00 log cfu/ml, respectively. Also Abdel Gadir et al. [1], Barbour et al. [8], Chaffer et al. [12], Semereab and Molla [39], Tuteja et al. [45] and Elgadi et al. [15] reported the presence of *Staphylicoccus* spp. in different camel milk samples. El- Ziney and Al- Turki [18] reported the mean count of S. aureus in camel milk samples as 2.74, while the highest level of contamination reached to 6.72 log cfu/ml, however S. aureus was detected in all tested samples in camel milk in Morrocco with an average of 5.1 log cfu/ml [9]. Obied et al. [32], Almaw and Molla [4], Sena [40] and Abdel Gadir et al. [1] reported that coagulase positive and negative staphylococci was frequently isolated from camels and can be considered as the main reason for mastitis in dromedaries.

A high count of *Staphylococcus aureus* presents a potential health hazard particularly for the presence of enterotoxigenic strains. The presence of enterotoxigenic strains in food does not always mean that the toxin will be produced, but it demonstrates the need for hygene thropught the stages of production.

The *Enterobacteriaceae* family includes *E.coli, Enterobacter, Citrobacter*, *Klebsiella*, *Salmonella, Shigella, Serratia, Yersinia, Morganella* and *Proteus,* which are isolated from animal intestines [13-24]. The existence of *Enterobacteriaceae* may not necessarily indicate direct fecal contamination of a camel milk or chal sample, but it shows evidence of poor sanitary practice during milking, handling and chal preparation. In the current study, mean Enterobacteriaceae counts were 2.19 and 1.88 with the minimum of 1.9 and 1.77 and the maximum of 2.6 and 2.04 for chal and camel milk samples respectively. El- Ziney and Al-Turki [18] reported mean count values of Enterobacteriaceae in camel milk as 2.7 with a maximum 6.82 log cfu/ml, and maximum total coliform value was 4.2 log cfu/ml. The total Enterobacteriaceae count in this study is in agreement with that reported in Semereab and Molla [39] and Benkerroum et al. [9] for camel milk tests in Ethiopia and Morroco.

Salmonella and *Shigella* organisms were not detected in any tested samples of chal, but existed in 8 of the camel milk samples. Presence of *Salmonella* in camel milk is in agreement with determinations in reports by El-Ziney and Al-Turki [17]. *Salmonella* infection is common in camels and other animals in countries worldwide. Fazil and Hofman [19], Wernery [49], Semereab and Molla [39] and Burgemeister [11] proved that the presence of *Salmonella* Typhimurium and *S.* Enteritidis in 5.8 % of the examined camels, and El-Ziney and Al-Turki [17] isolated salmonella in 24% of tested camel milk samples in Egypt.

Minimum and maximum total bacteria counts for camel milk (hand milking) reported by Valerie [45] 0.2×10^{1} – 4.2×10^{3}, Staphylococci, 3.9×10^{2}, Coliform, 2.8×10^{2} cfu/ml, and no salmonella and Bacillus Cereus was found.

El-Ziney and Al-Turki [17] found presence of salmonella spp. in 8 out of 33 samples, whereas Jayarao and Henning [25] reported that the isolation rate for this organism in raw cow milk within the range of 3-9%.

As yet there have been no reports of cases of transmission of salmonella from camels to humans from milk or milk products [52]. The most common reason for the presence of *Salmonella* spp. in milk is through fecal contamination after heat treatment because *Salmonella* are inactivated during pasteurization [27]. It is necessary to consider that chal prepared from raw milk and unpasteurized camel milk may contain presence the *Salmonella* and *Shigella* and this should not be considered as unusual. Intestinal *Salmonella* infections are occurring in livestock in Saudi Arabia and Kuwait, but no outbreaks of salmonellosis have been reported in animals in the UAE since 1996 [33], whereas *Salmonellae* have been isolated from healthy camels in the UAE [48].

Salmonella and *Shigella* were not found in any of the chal samples and this might be due to that fact that *Salmonella* and *shigella* are already in raw milk but are not in sour chal. AlsoVlaemynck [47] stated that contamination of raw milk with *Salmonella* and *Shigella* is usually from external sources. Presence of *Salmonella* and *Shigella* in camel milk in this study wasin agreement with reports in other studies by Deutz et al. [14], Sharmanova et al. [42] and Stephan and Buther [44] for cow milk.

As yet there are no regulations for microbiological standards for camel milk and its products, therefore, values for the microbiological limits for cow milk were used to asses the quality of camel milk.

4. Conclusions

The nature of fermented dairy produce is variable from one region to another. The present study concludes that the hygienic quality determined for camel milk was low so it recommended that milking be done under hygienic conditions and then the milk should be cooled immediately; it is also recommended that chal be produced under hygienic conditions in order to prevent contamination with undesirable microorganisms especially pathogenic microorganisms. In this study there were some undesirable or pathogenic microorganisms in chal samples, but further study is needed to detect toxins that are produced by *S.aureus*, *E.coli*, spore forming bacteria and other harmful microorganisms in chal and camel milk.

These results suggest that microbial contamination was affected by the different climate conditions in the countries involved in the various reports that were considered in related studies and that these influenced determinations of microorganisms.

This study recommends that improving hygiene practice in chal production would be an effective way to decrease yeast contamination, accordingly hand washing and udder cleaning before milking seems to be an effective method to decrease microbial contamination in milk. Also, the water used in cleaning operations and added to chal (at the preparation stage) should be good quality because the microbial quality of water has an affect on the hygienein camel milk and chal.

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