Journal of Animal and Veterinary Advances 10 (19): 2511-2515, 2011
ISSN: 1680-5593

Microflora and Pathogen Bacteria in Traditional Anatolian Canak (Pan) Cheese

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Abstract: In this study, a total of 35 samples, selected randomly from different canak cheese producers in Yozgat a province of Anatolia were examined for their conformity to Turkish Food Codex Microbiological criteria. The average counts of total aerobic mesophilic bacteria, *Lactococcus* sp., *Lactobacillus* sp., coliform bacteria, *Staphylococcus-Micrococcus* sp., *Enterococcus* sp., Enterobacteriaceae, yeast and mould were determined to be 7.48, 6.60, 6.79, 2.62, 3.97, 3.34, 3.32, 4.29 and 2.97 log cfu g^{-1}, respectively. Only 15 samples conformed to the Turkish Food Codex. Coagulase-positive staphylococci were detected in 24 out of 35 samples (68.5%) and *E. coli* was detected in 10 out of 35 samples (28.5%). According to the results of microbiological analysis, it was concluded that Canak cheese may carry a potential public health risk and therefore, strict hygiene precautions should be taken during production, storage and sale stages.

Key words: Cheese, Traditional cheese, quality, microbiological quality, Canak cheese, Turkey

INTRODUCTION

Turkey which had been the cradle of various civilizations over thousands of years has a geography very suitable for stock-breeding. It also offers a very rich variety of cheeses such as White cheese, Kashar cheese, Tulum cheese, Cokelek, Pan cheese and so on. There are actually >110 types of cheese in Turkey. According to the latest data, a total of approximately 3250 enterprises whose daily capacity ranges from several hundred liters to 10-15 tons are reported to be making and selling dairy products in Turkey. Of these enterprises, around 150 were modern. It has been estimated that 60% of the cheeses produced in Turkey are White cheese, 17% Kashar cheese, 12% Tulum and Mihalic cheese and that the remaining 11% of production is made up of other local cheeses. According to the Turkish Statistical Institute (TUIK), the country produces 12,329,789 tons of milk and 130,836 tons of cheese annually.

Canak (Pan) cheese which is matured underground is a common cheese variety native to the district of Sarikaya, Bogazlayan, Sorgun, Sefaatli, Cayiralan and Yerkoy in Yozgat province. Artisanal Canak cheese is produced by local traditional methods to meet family needs and consumed locally. Fermentation relies on the activities of indigenous milk microflora. The manufacturing practices of Canak cheese in the absence of any rigorous temperature and relative humidity control during storage, causes variations in the final quality of the product. This variation in Canak (Pan) cheese indicates the absence of any standardized method for the production of this cheese variety (Kamber and Terzi, 2008).

The color of Canak cheese is white. It has a homogenously granulated structure (Fig. 1). It can be classified as a semi-hard cheese and has a slightly salty taste. This cheese variety is usually produced during summer months (June, July and August) and is buried underground between September and November for full maturation (Kamber and Terzi, 2008).

Until now, research on Canak (Pan) cheese had been about the gross composition and production technology. Limited microbiological characterization of Canak cheese was also performed by Kirdar. Researchers reported the total aerobic mesophilic bacteria, coliform bacteria, Staphylococci, yeast and mould as 7.73±0.68, 2.59±0.83, 4.19±1.14, 4.33±0.76 and 3.15±0.84 log cfu g^{-1}, respectively. Very limited information is available in the literature on the microbiological characteristics of Canak cheese.

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Fig. 1: Canak cheese is produced map of the region

Canak cheese is made traditionally from raw goat or ewe's milk and no starter culture is used. Raw milk is coagulated at 32-35°C using home-made calf rennet (a quarter tea glass per 30 kg milk) for about 60-90 min. The curd is broken into four large pieces with a ladle and is rested 15-20 min. The curd is transferred into cambric cloth bags. After draining, pressure is applied to the curd (3-5 kg of weight for each kilogram of cheese) and kept like that for an hour. After draining, the curd is placed on the cloth in the tray, broken into pea-size pieces by hand and then salted. The cloth bag is placed on a rocky surface in a cool spot (12-16°C) and pressure is applied by using regular-shaped stones in such a way that there will be 10 kg of weight for each kilogram of cheese. The cheese is then either cut into slices like a potato or crumbled. The pans are filled in alternating layers of sliced and crumbled until they are full. Finally, the pan's mouth is closed and turned upside down. It is buried again in the sand but this time fully. Maturing is done in a cool and slightly humid environment such as a storeroom with the cheese being placed either directly in the ground or inside a box full of sand. The canak (Pan) is then ripened for 3 months (Kamber and Terzi, 2008).

The aims of this study were to determine the microbiological quality of traditional Canak cheese produced in Yozgat province of Turkey.

MATERIALS AND METHODS

The microbial profile of thirty-five ripened cheese samples (~200 g each) were collected in April-November 2009 from different producers in Yozgat province (especially Sorgun bazaar). The approximate age of the ripened cheeses was 3 months.

The samples were transported to the laboratory in an ice chest in sterile plastic bags under aseptic and refrigerated conditions at 5°C. Microbiological analysis was performed within 1-2 h after collection. Samples were analyzed in the Laboratory of Veterinary Faculty of Mehmet Akif Ersoy University. Microbiological analyses were carried out in triplicate.

Microbiological analyses: Representative 25 g cheese samples were homogenized using a Stomacher 400 with 225 mL of sterile 0.1% peptone water for at least 2 min. Decimal dilutions of the homogenized samples were prepared in sterile peptone water and plated in duplicates onto specific media.

The cheese samples were examined for total aerobic mesophilic bacteria, *Lactobacillus* sp., *Lactococcus* sp., *Staphylococcus-Micrococcus* sp., Enterobactericeae, coliform bacteria, *E. coli*, *Enterococcus* sp., yeast and mould. All selective media were prepared according to the manufactures' instruction.

Total mesophilic aerobic bacteria were counted on standard Plate Count Agar (PCA) incubated at 35±1°C for 48 h (Peeler and Maturin, 1992). Baird-Parker agar was used for counting of Staphylococci-Micrococci at 35±2°C for 48 h. Colonies were examined by Gram stain, catalase test, anaerobic utilization of glucose and mannitol and coagulase test (BAM, 2001). VRBD Agar (violet red bile dextrose agar) was used for isolation of Enterobacteriaceae at 37±0.1°C for 48 h. The count of total coliforms was performed on standard Violet Red Bile (VRB) agar incubated at 35±1°C for 24-48 h (APHA, 1976). From positive cultures, sub-cultures were made on Eosin Methylene Blue lactose sucrose (EMB) agar and incubated at 35±1°C for 24 h. *E. coli* isolates were biochemically characterized by IMVIC tests. Yeasts and

moulds were counted on Potato Dextrose Agar (PDA) (Oxoid) following the pour-plate method and incubated at 25°C for 5-7 days (BAM, 2001). Enterococci was grown on Slanetz-Bartley Agar plates (SBA) and subsequently incubated at 37±0.1 °C for 48 h (Facklam and Sahm, 1995). MRS agar was used for counting *Lactobacillus* sp. (Dupont *et al*., 2000) and M17 agar for *Lactococcus* sp. (Terzaghi and Sandine, 1975). Identification of the isolates was performed according to the criteria of Bacteriological Analytical Manuel (Anonymous, 2001). All media were obtained from Oxoid (Unipath Ltd., Basingstoke, England).

Statistical analyses: Statistical parameters including mean, standard error, range, standard deviation and correlation of variation were determined to estimate variability in the microbiological characteristics of Canak cheese. Microbial counts were expressed as the logarithm (log) of colony forming units (cfu) g^{-1} of cheese. All statistical analyses were performed using SPSS software running on Windows 9.05 platform.

RESULTS AND DISCUSSION

In this study, thirty-five mature Canak cheese samples were examined for total aerobic mesophilic bacteria, *Lactobacillus* sp., *Lactococcus* sp., *Staphylococcus-micrococcus* sp., Enterobactericeae, coliform bacteria, *E. coli*, *Enterococcus* sp., yeast and mould. The results obtained are shown in Table 1. The population distribution of the microorganisms is shown in Table 2 and the correlations between the various microorganisms are shown in Table 3.

The Total Aerobic Mesophilic bacteria (TAMB) counts ranged from 6.00 and 8.62 log cfu g^{-1} with the average of 7.48 log cfu g^{-1} (Table 1). The average count of coliform bacteria was 2.62 log cfu g^{-1}. The average count of *Staphylococcus-Micrococcus* sp. was 3.97 log cfu g^{-1} (Table 1). Enterococci counts ranged from 2.00-8.26 log cfu g^{-1} with the average of 3.34 log cfu g^{-1}. The average number of lactic acid bacteria (grown on M17 agar) was 6.60 log cfu g^{-1}, ranging from 4.08-7.85 log cfu g^{-1} and lactic acid bacteria (grown on MRS agar) was 6.79 log cfu g^{-1} ranging from 6.00-8.60 log cfu g^{-1}. The average count of Enterobactericeae 3.34 log cfu g^{-1}. The yeast counts ranged from 2.00-6.08 log cfu g^{-1} with the average of 4.29 log cfu g^{-1}. The average mould counts determined was from 2.97 log cfu g^{-1} cheese changing between 2.00 and 4.78 log cfu g^{-1} (Table 1).

The results were pointed out that high TAMB content of Canak cheese samples was due to the poor quality of raw milk used. Similar findings were reported for other types of traditional cheeses (Hamed *et al*., 1992; Al-Tahiri, 2005; Kivanc, 1989). TAMB counts of Canak cheese were lower than those for Carra cheese, Herby cheese, Tulum (Savak) cheese and White cheese which are other ripened Turkish cheese varieties made from unpasteurized milk (Aygun *et al*., 2005; Sengul *et al*., 2001).

In this research, it was shown that Canak cheese was contaminated with coliform bacteria after processing. The major factors raising the risk of contamination were: the use of raw milk, lack of pasteurization, poorly controlled natural fermentations inadequate storage conditions and the use of non-hygienic equipment. The Turkish Food Codex mandates that dairy products must contain no >100 cfu g^{-1} coliform bacteria and they must exclude *E. coli* (Anonymous, 2001). Of 35 Canak cheese samples investigated, only 15 (42.86%) samples conformed to the Codex. *E. coli* was detected in 10 out of 35 samples (28.5%).

Most food intoxications caused by *S. aureus* are the result of bad hygienic practices in household and industrial cheese production (Sengul, 2006). Canak cheese made from raw milk was contaminated with *S. aureus* ranging from 10^2-10^5 cfu g^{-1}. Only 15 (42.8%) out of 35 canak cheese samples analyzed conformed to the Turkish Food Codex (Table 2) (Anonymous, 2001). Coagulase-positive staphylococci were detected in 24 samples out of 35 samples (68.5%). The *S. aureus* counts in canak cheese were also higher than other traditional cheeses native to Turkey including Turkish white cheese (1.30-1.70 log cfu g^{-1}), Herby cheese (0.95 log cfu g^{-1}) and Tulum cheese (2.52 log cfu g^{-1}) (Aygun *et al*., 2005; Sengul *et al*., 2001).

Table 1: Presence of microorganisms in the Canak cheese samples (log cfu g^{-1})

Microbiological properties (log cfu g^{-1})	n[a]	X[b]	Statistical parameters ----------Range----------		SD[d]	SEM[e]
Total aerobic mesophilic bacteria	35	7.48	6.00	8.62	0.69	0.11
Lactococcus sp.	35	6.79	5.40	7.95	0.72	0.12
Lactobacillus sp.	35	6.60	4.08	7.85	0.84	0.14
Staphylococcus-Micrococcus sp.	35	3.97	2.00	5.95	1.34	0.22
Enterococcus sp.	35	3.34	2.00	8.26	1.59	0.26
Enterobacteriaceae	35	3.34	2.00	5.53	1.02	0.17
Coliform bacteria	35	2.62	2.00	5.00	0.76	0.12
Yeast	35	4.29	2.00	6.08	0.97	0.16
Mould	35	2.97	2.00	4.78	0.87	0.14

[a]Number of sample analyzed; [b]Mean; [d]Standard deviation; [e]Standard error of mean

Table 2: Population distribution of the microorganisms of Canak cheese samples (%)

	Percentage of samples in different population groups							
Microorganisms	<2	2-3	3-4	4-5	5-6	6-7	7-8	8-9
Total aerobic mesophilic bacteria	-	-	-	5.71	2.85	20.00	40.00	31.42
Lactobacillus sp.	-	-	-	-	22.86	40.00	37.14	-
Lactococcus sp.	-	-	-	5.71	11.43	48.57	34.29	-
Staphylococci-Micrococci	22.86	5.71	8.57	40.00	22.86	-	-	-
Coliform bacteria	42.86	34.28	20.00	2.86	-	-	-	-
Enterobactericiae	25.71	5.71	40.00	25.71	2.86	-	-	-
Enterococci	42.86	14.28	5.71	20.00	17.14	-	-	-
Yeast	2.86	2.86	20.00	54.29	17.14	-	2.86	-
Mold	17.14	34.28	40.00	8.57	-	-	-	-

Table 3: Correlation between the microorganisms of canak cheese samples

Microorganism (log cfu g^{-1})	TAMB	*Lactoc.* sp.	*Lactob.* sp.	Coliform	Enterobac.	Stap-Micro.	Enterococ.	Yeast	Mould
TAMB	1	-	-	-	-	-	-	-	-
Lactococcus sp.	0.688 (**)	1	-	-	-	-	-	-	-
Lactobacillus sp.	0.735 (**)	0.663 (**)	1	-	-	-	-	-	-
Coliform	0.101	0.025	0.130	1	-	-	-	-	-
Enterobactericeae	-0.153	-0.136	-0.124	0.745 (**)	1	-	-	-	-
Staphylococcus-micrococcus	0.193 (*)	0.197 (*)	0.112	0.382 (**)	0.368 (**)	1	-	-	-
Enterococcus	0.569 (**)	0.626 (**)	0.427 (**)	0.027	-0.155	0.127	1	-	-
Yeast	0.260 (**)	0.129	0.351 (**)	-0.052	-0.118	0.162	-0.101	1	-
Mould	0.481 (**)	0.288 (**)	0.436 (**)	-0.002	-0.150	0.098	0.172	0.392 (**)	1

$p<0.05$; ** $p<0.01$

Enterococci were found in a variety of artisanal cheeses made from raw or pasteurized milk from goats, sheep, water buffaloes and cows indicating poor sanitary conditions (Manolopoulo *et al*., 2003).

Enterobactericeae counts in the samples of cheese must be $<10^2$ g^{-1} in 25% of the samples with maximum 10^5 cfu g^{-1} (1 sample) for Canak cheese. The presence of Enterobactericeae in the cheeses may be due to the use of raw milk and/or contamination of the product during manufacturing from the environment and/or from a person (Giraffa, 2002). The correlation coefficient between the numbers of coliform bacteria and Enterobactericeae and *Staphylococcus-Micrococcus* sp. was r = 0.745 ($p<0.01$) and r = 0.382 ($p<0.01$), respectively (Table 3). A large variation was observed between samples which could be related to the production and ripening under conditions not suitable for good microbiological quality.

Lactic Acid Bacteria (LAB) count plays a fundamental role in ripening of cheese due to lactic acid fermentation developing required characteristics of dairy products. However, it is hardly possible to prevent contamination of coliform bacteria and especially proteolytic microorganisms into the cheese (Sengul, 2006; Stiles and Holzapfel, 1997). Previous studies showed the predominance of lactococci during the early stages of ripening (Litopoulou-Tzanetaki and Tzanetakis, 1992; Centeno *et al*., 1996; De Boer and Kuik, 1987). There was a significant positive correlation between TAMB and LAB counts ($p<0.01$) (Table 3).

According to the results obtained, the yeast and mould counts were found to be higher than the limits (<100 cfu g^{-1}) presented in Turkish Food Codex. The results indicated that Canak cheese was contaminated with yeast and mould after processing. The routine methods of controlling yeast and moulds in the Canak cheese include regular disinfectant wash down and fogging (Beresford *et al*., 2001).

In this research, the counts TAMB, coliform bacteria, *E. coli*, yeast, mould and presence *S. aureus* in Canak cheese were higher than the upper limits given in Turkish Food Codex. It is not surprising to obtain high microbiological counts in cheese with artisanal manufacturing methods due to the use of unpasteurized milk.

CONCLUSION

The study clearly showed that Canak cheese cannot be accepted as safe. It was found to contain high variations in its microbiological characteristics. Artisanal Canak cheese shows that it is produced primarily by hand in small batches with particular attention paid to the tradition of the cheese maker's art and thus using as little automation as possible in the production of the cheese. This product can be modernized from its production to consumption.

To improve the microbiological quality of canak cheese, pasteurized milk should be replaced with raw milk and the processing, ripening and marketing should be carried out under good hygienic conditions. It is essential

to develop a specific starter culture for this cheese type. This requires a complete microbiological and biochemical study of the artisanal Canak cheese, identifying the main microbial species present in the cheese and correlating their metabolic activities with the biochemical changes that occur during ripening.

ACKNOWLEDGEMENTS

The researchers thank Sefa Serdar Ornek for collection of cheese samples.

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