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# Bacterial contamination in fresh white cheeses sold in bazaars Canakkale, Turkey

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**Abstract**

Sixty specimens of fresh white cheeses, presented for sale in district bazaars at the center and in some towns of Canakkale, were analyzed microbiologically in this study. The Total Aerobic Mesophilic Bacteria (TAMB), *Escherichia coli*, *Pseudomonas* spp., *Stahpylococcus aureus*, *Salmonella* spp. and *Brucella* spp. bacteria, included in the specimens collected between February and October, were investigated. In the evaluation conducted, it was found out that the specimens contained TAMB at the interval of $5.2 \times 10^4 - 5.68 \times 10^{11}$ cfu/g and, in all collected specimens, Coliform group of bacteria and *E. coli* were isolated and the bacterial counts were found to be at the intervals of $1.0 \times 10^3 - 9.58 \times 10^8$ and $1.2 \times 10^2 - 3.6 \times 10^8$ cfu/g, respectively. *Salmonella* spp. were detected in 2 specimens and no *Pseudomonas* spp. and *S. aureus* bacteria were encountered. The *Brucella* spp. bacteria were not encountered in any of the specimens. In conclusion, it was observed that the specimens of fresh white cheese included in this study contained microbial contamination at a health-threatening level and did not comply with the related standards.



## Introduction

Milk and milk products are among the important food substances for every age group due to the nutrients they contain (Torres-Vitela *et al*., 2012). The milk, which is milked from the teats of a healthy animal, contains very low levels of microorganisms. Since the milked milk is a medium, where the microorganisms that are contaminated during milking, transporting and presenting for sale can develop easily, the foods, which are produced and marketed under unsuitable hygienic and sanitation conditions, cause infections or intoxications in human beings (Shrestha *et al*., 2011). The microorganisms, which infect through various sources, exhibit a very rapid growth in cheese, a nutrient that is quite rich in protein and calcium (Evrendilek *et al*., 2008). Today a considerable amount of milk produced in our country is produced in dairies that are deprived of technical information and modern instruments and by village women. Although classical methods are used generally in transforming milk into cheese, there are some application differences. The primary differences are pasteurization, coagulation (yeast addition) and maturation periods. Not pasteurizing the milk, not complying with hygienic rules and presenting cheese in fresh form for consumption without maturation are among the most important reasons that affect TAMB (total aerobic mesophilic bacteria) count (Hayaloglu and Kirbag, 2007). Coliform bacteria constitute the most dangerous group among the microorganisms that infect cheeses through various sources (Giammanco *et al*., 2011). The animal skin, the air in the barn, the cleanness of the containers used, the microorganisms that infect milk through the milker. The count of coliform group of bacteria is found to be high particularly in fresh white cheeses that are produced particularly at small family enterprises under primitive conditions and that are presented for sale in open form in district bazaars. *E. coli* is the most frequently observed microorganism among the microorganisms causing food intoxications in our country (Hayaloglu and Kirbag, 2007). In regions, where nonpasteurized milk and milk products are consumed, *Brucella* spp are also among the frequently-observed agents. Some of the cheeses made of goat milk in our country create great risk due to insufficient pasteurization (Ongör *et al*., 2006).

Pasteurization is a method that is generally used for milk. It was defined for the first time by Franz von Soxhlet in 1886. Today two different methods

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are applied widely: HTST (High Temperature Short Time) and UHT (Ultra High Temperature). In the method of HTST, milk passes through metal pipes, around which hot water passes, and is kept for 15 to 20 seconds at 71.7°C whereas, in the method of UHT, the process is completed in one second at 138°C. In our country, the processes of pasteurization are not carried out efficiently particularly in small and medium-sized manufacturing sites. Thus, bacterial contamination is observed frequently in the cheese produced especially by these enterprises and generally sold in bazaars. This study was conducted in order to determine the microbiological quality of fresh white cheeses that are presented for sale in open form in district bazaars of Canakkale region, the production conditions of which are unknown, and that are presented for sale under unsuitable hygienic conditions.

## Material and Method

This study was carried out with the specimens of fresh white cheese presented for sale in district bazaars set at the center of Canakkale and in its towns of Bayramic, Ezine and Lapseki between February and October. In our area, fresh white cheese was manufactuared originally from sheeps' and goats' milk. Most of them produced on village farms and also sold without storage. Because of this situation long lasting sampling period was preferred. This study was settled for screening; 15 specimen from four different bazaars of town randomized in nine months. Specimen collection time was randomized market places' settling day. From 100 grams of 60 cheese specimens bought under aseptic conditions, 10 grams were weighed from each under aseptic conditions and 90 ml of 1/4 strength Ringer's solution was added and series dilutions of up to $10^{-10}$ of the cheese specimens were made after homogenization. 0.1 ml of specimens, collected from dilutions, was cultured with spread method in the Plate Count Agar (MERCK 1. 05463) culture media (ISO, 1986). The growth in the plaques following incubation for 24 hours at 37°C was evaluated in terms of Colony forming unit (cfu). The dilutions prepared up to $10^{-8}$ from each specimen after homogenization were cultured by spread method on Violet Red Bile Agar, EMB Agar, Pseudomonas Selective Agar and Baird-Parker Agar culture media so as to search for coliform bacteria, *E. coli*, *Pseudomonas* spp. and *Stahpylococcus aureus* bacteria (ICMSF, 1982). Violet Red Bile Agar culture medium was used in finding out coliform bacteria and the plaques were incubated for 24 hours at 37°C. The typical red colonies, being 1-2 mm in diameter, which developed in the culture media, were Gram-stained and these bacteria were identified using the other methods required (ICMSF,1982). For the isolation of *E. coli*, EMB Agar was used and the plaques were incubated for 24 hours at 37°C. The IMVIC test was applied to the colonies with greenish metallic luster and the colonies, which gave positive results, were evaluated as *E. coli*. For the isolation of *Pseudomonas* spp., the Pseudomonas Selective Agar (MERCK 1.05284) culture medium was cultured and incubated for 48 hours at 35-37°C. In order to search for *S. aureus*, a tube coagulase test was made by Coagulase Plasma EDTA (DIFCO 0803-46-5) with the atypical colonies growing after incubation for 24 hours at 37°C in Baird-Parker Agar culture medium(ICMSF,1982). For the isolation of *Salmonella* spp., 25 grams of cheese was weighed from each cheese specimen and homogenized for 1 minute in blender with 225 ml of buffered Peptone water (MERCK 1.07228). Following pre-enrichment, 0.1 ml was collected from each specimen and 10 ml from each were cultured in Rappaport-Vasilliadis Broth. They were incubated for 24 hours at 43°C and selective enrichment was performed. Following this procedure, Brilliant green Agar was cultured and the plaques were incubated for 24-48 hours at 37°C. The presence of lactose-negative pink-red colonies having grown in BPLS Agar was investigated (ICMSF, 1982).

For *Brucella* spp., 0.1 ml of suspension from the homogenized cheese specimens was cultured in Brucella selective culture medium. It was prepared by adding Brucella Supplement (OXOID SR 0083A) to the Brucella culture medium (MERCK 1.10490). The plaques were incubated for 4 to 5 days at 37°C in an aerobic medium with 10% $CO_2$ and the grown atypical colonies were examined macroscopically as well as microscopically by gram staining technique (ICMSF, 1982).

## Results

It was detected that the specimens of fresh white cheese contained TAMB at the interval of $5.2x10^4$ and $5.68x10^{11}$ cfu/g. Coliform bacteria and E. coli bacteria were isolated from all of 60 specimens of fresh white cheese collected from the district bazaars at the center and in the towns of Canakkale and included in the study. In the specimens collected, the counts of the Coliform group of bacteria were detected to be between $1.0x10^3$ and $9.58x10^8$ cfu/g and the bacterial count of *E. coli* was found to be at the interval of $1.2x10^2$ - $3.6x10^8$ cfu/g. *Salmonella* spp. were isolated in 2 of the specimens examined. The

*Pseudomonas* spp. and *S. aureus* bacteria could not be isolated from the specimens. Brucella bacterium wasn't encountered in any of the specimens that were taken to Brucella Selective Agar culture medium and left for incubation at 37°C for 4-5 days in aerobic and anaerobic media for examination in terms of *Brucella* spp.

## Discussion

In the traditional or artisanal manufacture of Turkish White cheese, starter culture is not added to the cheesemilk. The milk may or may not be pasteurized and the curd is handled extensively by the cheese maker (Hayaloglu *et al*., 2002). At almost every stage of cheese production technology, there are risk factors that are likely to cause microbial contamination. There are many factors that affect the total aerobic mesophilic living bacteria count in foods. Not pasteurizing the milk, with which cheese will be made, not complying with hygienic rules, and presenting cheese in fresh form for consumption without maturation affect microbiological quality. As a matter of fact, it has been shown that the total TAMB count found to be less in cheeses made of pasteurized milk than in cheeses made of raw milk. Similar results were obtained in the specimens of Turkish cheese presented for sale (Turantas *et al*., 1989: Karakus and Alperden, 1992; Temelli *et al.*, 2006).

In our study, the TAMB count in the specimens of fresh white cheeses sold in district bazaars was detected to be at the interval of $5.2x10^4 – 5.68x10^{11}$ cfu/g and the mean TAMB was found to be $3.7 \times 10^{10}$ cfu/g. This result is higher than the similar studies although slightly (Temelli *et al*., 2006). It is known that there is a decline in TAMB count upon an increase in the maturation period of cheeses (Manolopoulou *et al*., 2003). Numbers of microorganisms indicative of the hygienic quality, such as coliforms, enterococci and staphylococci were present in cheese at relatively high levels. The high TAMB count in this study in comparison to standards provokes one think that the specimens examined were presented for sale before the completion of their maturation period. As it is known, coliform group of bacteria is quite high in human and animal faeces. Its infection to foods demonstrates that that food is exposed to a faecalborne infection. The differences between the analysis results of fresh white cheese specimens in this research and the values reported by other researchers may be either due to the variations in productions, storage conditions and durations of the examined specimens or due to the difference in the methods applied.

Human brucellosis is the most common bacterial zoonosis. Syria, Mongolia, Kyrgyzstan, Iraq and Turkey have the highest incidence world-wide (Pappas et al., 2005). Contamination of cheese especially made from goat milk has a critical role in Brucellosis. In our country, molecular and conventional microbiologic studies showed *Brucella* spp. in cheese (Kasimoglu A, 2002; Ongor *et al*., 2006: Alim and Tomul 2005). *Brucella* spp. wasn't encountered in any of 60 fresh white cheese specimens examined in this study.

Turkish white cheese is usually produced under unmechanised conditions. Various type of microorganisms may enter the cheese during manufacture and subsequent handling (Turantas *et al*., 1989). In the 60 cheese specimens examined in this study, the coliform bacterial count was detected to be between $1.0 \times 10^3$ and $9.58 \times 10^8$ cfu/g and the *E. coli* bacterial count was found to be at the interval of $1.2 \times 10^2 – 3.6 \times 10^8$ cfu /g. According to the Turkish Food Codex, maximum possible coliform bacterial count in cheese should be 95 cfu/ g. In addition, there should be no *E. coli* or *Salmonella* spp in cheese (Turkish Food Codex, 2011).

According to our knowledge there wasn't any study in the literature that showed *Salmonella* spp. in cheese sold at markets. In two cheese samples *Salmonella* spp. was isolated. The results obtained in the research demonstrate that the hygienic quality of fresh white cheeses sold in district bazaar in Canakkale is low and does not have enough assurance in terms of public health. In order to correct this quality, it is of extreme importance that the animal barns and the medium, where milk is processed, comply with health rules besides that attention should be paid to the cleanness of the animal teats, milker and the materials used in the process of milking. It was once more understood that it is absolutely required to apply processes such as hygiene, pasteurization, maturation and etc. in the period from milking process to its presentation for sale as milk products. It was once more appeared that it is required not to produce cheese from raw milk and to store it for minimum 90 days and under conditions of <10°C with a convenient concentration.

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