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Evaluation of the Principle Microbiological Flora of Cheeses at Retail Sale in Bazaars of Çanakkale

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Abstract

Food contamination, either from microbiological or chemical, is the highest concern for public health. The dairy products such as cheese have an essential role in human nutrition but have been linked with outbreaks of food-borne illness. The cheese production process contains the steps that might be the reasons of bacterial pathogens contamination. The primarily purpose of this study was to detect the microbiological quality and Lactic Acid flora of cheeses at retail sale in bazaars of Bayramiç, Ezine and Kepez (towns of Çanakkale) which were produced at home or small dairies. The presence of Total Mesophilic Aerobic Bacteria (TMAB), Total Psychrophilic Aerobic Bacteria (TPAB), yeast and moulds, coliform bacteria, coagulase positive *Staphylococcus spp.*, and *Escherichia coli* O157:H7 (EHEC) were investigated. The mean TMAB counts were assessed as 3.63×10^8 cfu/g (2.00×10^6- ≥ 1.00×10^9 cfu/g), 2.19×10^8 cfu/g (1.00×10^4- ≥ 1.00×10^9 cfu/g), and 1.55×10^8 cfu/g (2.00×10^6- ≥ 1.00×10^9 cfu/g) in cheese samples from Bayramic, Ezine and Kepez, respectively. TPAB was not determinated. The mean counts of coliforms were determinated as 1.44×10^5 cfu/g (ND- ≥ 1.00×10^6 cfu/g), 1.04×10^5 cfu/g (ND- ≥ 8.00×10^5 cfu/g) and 5.71×10^1 cfu/g (ND- ≥ 8.00×10^2 cfu/g) in cheese samples from Bayramiç, Ezine and Kepez respectively. *E.coli* 0157:H7 and coagulase positive *Staphylococcus aureus* were not detected. The average colony counts of *Lactococcus spp.*, *Enterococcus spp.* and *Lactobacillus spp.* were detected as 2.07×10^8 cfu/g (ND- ≥ 1.00×10^9 cfu/g), 2.43×10^6 cfu/g (ND- ≥ 3.30×10^7 cfu/g) and 1.54×10^8 cfu/g (ND- ≥ 1.00×10^9 cfu/g) in town Bayramiç; 1.86×10^8 cfu/g (ND-1.00×10^9 cfu/g), 1.46×10^5 cfu/g (ND-6.1×10^6 cfu/g) and 1.31×10^8 cfu/g (ND- ≥ 1.00×10^9 cfu/g) in town Ezine; 9.57×10^7 cfu/g (ND- ≥ 1.00×10^9 cfu/g), 1.23×10^5 cfu/g (ND-1.00×10^6 cfu/g) and 8.24×10^7 cfu/g (ND- ≥ 1.00×10^9 cfu/g) in town Kepez, respectively. According to this study results, cheese samples produced without uncontrolled conditions such as in small dairy farms or home and sold in bazaars of Çanakkale have high microorganism loads and do not meet the microbiological criteria in Turkish Food Codex regulations.

Keywords: Microbiological quality; Lactic acid flora; Home-made cheeses; Small dairy cheeses; Retail sale in bazaar; Çanakkale; Turkish Food Codex regulations

Introduction

As a fermented milk product, cheese production process evolved during centuries as a means of preserving raw milk by beneficial natural or starter flora such as *lactobacilli*, *streptococci* and *lactococci* [1]. Although, cheese production process in the world includes coagulating milk with rennet, filtering, shaping, brining and ripening steps at different periods; differentiated according to the particular demands of people, cultures and climate of each country [2,3]. The estimated number of different number of cheese type is around 1400 in all over the world. Among them, more than 110 of these different cheese types are produced in Turkey in which they are also differentiated according to various production techniques in different regions [2,4]. In spite of essential role in human nutrition, dairy products such as cheese have been linked with outbreaks of food-borne illness. The cheese production process that is discussed above contains the steps that might be the reasons of bacterial pathogens contamination; poor microbiological quality of the cheese-making milk such as the presence of pathogens in the raw milk and the survival of these pathogens in the process, hygienic drawbacks of manufacturing facilities, and the poor quality of packaging and handling of cheese are the examples of pathogens contamination. Furthermore, bacterial pathogens can contaminate cheese *via* post-processing contamination due to lack of sanitation, poor re-contamination measures and improper retail sale in street shops or bazaars [1,5]. Many studies have documented that the pathogens such as *Salmonella enterica*, *Listeria monocytogenes*, *Staphylococcus aureus* and enteropathogenic *Escherichia coli* (ETEC) pose greatest risk to the safety of cheese [1,5,6]. Microbial growth of pathogens in cheese is influenced by intrinsic parameters such as pH and acidity, nutrient and salt content, water activity (aw), redox potential, presence of antimicrobial compounds, either those occurring naturally or those which are added as food preservatives or by extrinsic parameters including packaging atmosphere, temperature and moisture conditions of storage [1,7]. Initial quality of raw milk is important in the production process of all kinds of cheeses. Even if the presence of small numbers of bacteria particularly *Enterobacteriaceae* and somatic cell is considered to be admissible in raw milk, increase in the number is indicated as poor husbandry, poor hygiene practices or bad preservation during milk collection. It also leads to increase in risk for pathogen contamination of cheese [8]. Even though majority of spoilage microorganisms from low quality raw milk, is eliminated during pasteurization, thermoduric species such as *Bacillus*, *Clostridium*, *Lactobacillus*, *Microbacterium*, *Micrococcus* and *Streptococcus* can survive in milk after heat treatment [7], and many

contaminants from processing, packaging and marketing environments including biofilm producer *Listeria spp.* can lead to post-pasteurization [1]. Variety of cheese also enhances or inhibits the growth of bacterial pathogens. Although hard and semi-hard cheeses restrict the growth of bacteria pathogens by their relatively low contents and pH values, high moisture and neutral pH of soft cheese varieties such as Camembert, facilitate growth of pathogens. For the production of safety foods, many regulations have been developed by several regulatory agencies. Control requirements include personnel health and cleanliness, sanitation practices in the plant process, water supplies, sanitation of equipment and establishment of production and process controls [3]. The production (at home and/or small dairy farms) and marketing of foods (in bazaars) which does not take care of these principles of the programs such as HACCP may lead to several foodborne illnesses. According to WHO's 2015 reports, 31 foodborne hazards causing 32 diseases and these hazards caused 600 million foodborne illnesses and 420.000 deaths in 2010. Diarrhoeal disease agents, particularly norovirus, non-typhoidal *Salmonella enterica* and *Campylobacter spp.*, get ranked first among the foodborne illness and also caused 230.000 deaths [9].

The primarily purpose of this study was to detect the microbiological quality and lactic flora of cheeses at retail sale in bazaars of Bayramiç, Ezine and Kepez (towns of in Çanakkale), which were produced by using goat, sheep or cow milk without any additional starter culture at home and/or small dairy farms. The presence of Total Mesophilic Aerobic Bacteria (TMAB), Total Psychrophilic Aerobic Bacteria (TPAB), yeast and moulds, coliform bacteria, coagulase positive *Staphylococcus spp.* and *Escherichia coli* O157:H7 (EHEC) was investigated, as suggested by the legislation on food security. Counts of *Lactobacillus spp.*, *Lactococcus spp.* and *Enterococcus spp.* were investigated in this study which is considered as members of Lactic Acid Bacteria (LAB). While the counts of bacteria belonging to the families Enterobacteriaceae and Staphylococcaceae are considered as indicators of milk quality and hygiene of the production process and sale conditions, lactic flora is known as the responsible for the ripening of non-starter cheeses.

Materials and Methods

Sampling

Thirty eight home-made cheese samples, were produced by using goat, sheep or cow milk without any additional starter culture, were randomly collected from bazaars in the towns of Bayramiç, Ezine and Kepez in Çanakkale/Turkey. The samples (at least 100 g) were taken using aseptic techniques and transferred to the Çanakkale Onsekiz Mart University, Bayramiç Vocational College, Food Technology laboratory under cold chain (4°C). The samples were examined for the presence of the following groups of microorganisms: Total Mesophilic Aerobic Bacteria (TMAB), Total Psychrophilic Aerobic Bacteria (TPAB), yeast and moulds, coliform bacteria, coagulase positive *Staphylococcus spp.*, *Escherichia coli* O157:H7 (EHEC), *Lactobacillus spp.*, *Lactococcus spp.* and *Enterococcus spp.*

Microbiological analysis

Ten grams of each cheese sample was aseptically homogenized with 90 ml sterile ¼ Ringer solution (Merck, Germany) for 2 min in the Stomacher (AES-EasyMix) and decimal dilutions (up to 10-6) of samples were prepared using same solution. For quantitative detection of microorganisms appropriate amounts from the ten-fold dilutions were pour-plated or surface plated parallel on to appropriate media according to standard microbiological methods. For the enumeration of TMAB and TPAB Plate Count Agar (PCA, Merck, Germany) were used and colonies were counted after 48 h of aerobically incubation at 30°C and 10 days of incubation at 6.5°C, respectively. Yeast and moulds was enumerated after 5 days (25°C) of inoculation of Yeast Extract Glucose Chloramphenicol Agar (YEGC Agar, Merck, Germany). Violet Red Bile Agar (VRB Agar, Merck, Germany) was used for the count of coliform bacteria, and after incubation of double layer pour plates (for 24 h at 37°C) the red violet coloured colonies with 2-3 mm diameter were evaluated as coliform bacteria. For detection of coagulase positive *Staphylococcus spp.*, Baird Parker Agar (BP Agar, Merck, Germany) with Egg Yolk Tellurite Emulsion was used. Inoculated plates by surface plague method were incubated aerobically at 37°C for 48 h. The dark grey to black, shiny, convex and 1-1.5 mm diameter (due to tellurite reaction)and surrounded by a clear zone which may be partially opaque, with/without an opalescent ring in around this clear zones (due to egg yolk reaction) colonies and the weak, grey to black colonies free of clear or opaque zones were counted as *Staphylococcus spp.* The coagulase test was also performed to the selected doubtful typical colonies (surrounded by a clear zone), positive result was approved as coagulase positive *Staphylococcus aureus*.

For detection of *Escherichia coli* O157:H7 (EHEC) suspensions from appropriate dilutions were surface plated on to Sorbitol-MacConkey Agar (SMAC Agar, Merck, Germany)with CT (Cefixime Tellurite) supplement, incubated at 37°C for 24 h in aerobically conditions. The colourless colonies were numerated as *E. coli* O157:H7 due to *E. coli* O157:H7 (EHEC) is sorbitol negative. For the confirmation of *E. coli* O157:H7 IMVIC (Indol, Methyl red, Voges Proscauer and Citrate) tests were also performed to the 5 randomly selected typical colonies. Rogosa Agar and Kanamycin Aesculin Azide Agar (KAA) (Merck, Germany) were used for selective enumeration of *Lactobacillus spp.* and *Enterococcus spp.,* respectively. Double layer inoculated Rogosa plaques by surface plate method were incubated at 37°C for 24-48 h and cream coloured colonies were counted as *Lactobacillus spp.*; after incubation at 37°C for 24-48 h the olive-green-black colonies surrounded by a dark zone on KAA were counted as *Enterococcus spp.* Enumeration of *Lactococcus spp.* were performed on M17 Agar (Merck, Germany) after incubation at 30°C for 24-48 h.

The plates with 10-300 typical bacterial colonies were selected for enumeration and the number of colonies developed in each medium was expressed as cfu g^{-1}. Gram staining, catalase and oxidase tests were performed to the 5 typical colonies which are randomly selected from each enumerated plate. All groups of microorganisms, each mediums, incubation conditions and confirmation tests are shown in Table 1.

Microorganisms	Mediums	Incubation Conditions	Confirmation Tests*
Total Mesophilic Aerobic Bacteria (TMAB)	Plate Count Agar (PCA)	30°C for 48 h	—
Total Psychrophilic Aerobic Bacteria (TPAB)	Plate Count Agar (PCA)	6.5°C for 10 d	—
Yeast and moulds	Yeast Extract Glucose Chloramphenicol Agar (YEGC Agar)	25°C for 5 d	—

Coliforms	Violet Red Bile Agar (VRB Agar)	37°C for 24 h	—
Staphylococcus spp.	Baird Parker Agar (BP Agar) with Egg Yolk Tellurite Emulsion	37°C for 48 h	Gram staining, Catalase test, Oxidase test, Coagulase test
Escherichia coli, O157:H7 (EHEC)	Sorbitol-MacConkey Agar (SMAC Agar) with CT supplement	37°C for 24 h	Gram staining, Catalase test, Oxidase test, IMVIC (Indol, Methyl red, VogesProscauer and Citrate) tests
Lactobacillus spp.	Rogosa Agar	37°C for 24-48 h	Gram staining, Catalase test, Oxidase test
Lactococcus spp.	M17 Agar	30°C for 24-48 h	Gram staining, Catalase test, Oxidase test
Enterococcus spp.	Kanamycin Aesculin Azide Agar (KAA)	37°C for 24-48 h	Gram staining, Catalase test, Oxidase test

Table 1: Microorganisms groups, mediums, incubation conditions and confirmation tests.

Results and Discussion

To evaluate the microbiological quality of home or small dairy farm made cheeses which were exposed to sale in bazaars of Bayramiç, Ezine and Kepez (Çanakkale), thirty eight samples were investigated and the microbiological counts, means (cfu/g)and standard deviations (SD)of each towns are presented in Tables 2-4 respectively.

According to the microbiological analysis the mean TMAB counts were assessed as 3.63×10^8 cfu/g (2.00×10^6- $\geq 1.00 \times 10^9$ cfu/g), 2.19×10^8 cfu/g (1.00×10^4- $\geq 1.00 \times 10^9$ cfu/g), 1.55×10^8 cfu/g (2.00×10^6- $\geq 1.00 \times 10^9$ cfu/g) and mean Yeast and Moulds counts were assessed as 4.94×10^5 cfu/g (ND- $\geq 1.00 \times 10^6$ cfu/g), 2.42×10^5 cfu/g (ND- $\geq 1.00 \times 10^6$ cfu/g), 1.63×10^5 cfu/g (ND- $\geq 1.00 \times 10^6$ cfu/g) in cheese samples from Bayramiç, Ezine and Kepez, respectively. TPAB was not determinated in each town (data not shown).

The mean counts of coliforms were determinated as 1.44×10^5 cfu/g (ND- $\geq 1.00 \times 10^6$ cfu/g); 1.04×10^5 cfu/g (ND- $\geq 8.00 \times 10^5$ cfu/g); 5.71×10^1 cfu/g (ND- $\geq 8.00 \times 10^2$ cfu/g) in towns Bayramiç, Ezineand Kepez, respectively. In this study doubtful *E. coli* 0157:H7 colonies (colourless) were not detected (data not shown).

Sample No	TMAB	Yeast and Moulds	Coliform	*Staphylococci spp.*	*Lactobacillus spp.*	*Lactococcus spp.*	*Enterococcus spp.*
B1	2.0×10^6	$\geq 1.0 \times 10^6$	ND	3.08×10^4	ND	9.5×10^6	ND
B2	1.25×10^7	$\geq 1.0 \times 10^6$	3.0×10^3	2.67×10^4	ND	1.1×10^7	ND
B3	2.455×10^8	$\geq 1.0 \times 10^6$	1.16×10^5	1.0×10^7	3.0×10^6	1.06×10^8	1.0×10^6
B4	4.4×10^7	$\geq 1.0 \times 10^6$	ND	1.0×10^7	2.0×10^6	1.0×10^6	ND
B5	6.39×10^8	$\geq 1.0 \times 10^6$	1.18×10^5	3.28×10^5	3.2×10^7	3.53×10^8	3.3×10^7
B6	5.7×10^7	2.01×10^4	ND	8.0×10^4	3.9×10^7	1.11×10^8	1.2×10^6
B7	2.11×10^8	$\geq 1.0 \times 10^6$	ND	3.9×10^5	3.7×10^7	2.24×10^8	2.55×10^5
B8	1.23×108	ND	ND	1.0×10^4	2.13×10^7	6.01×10^7	3.0×10^4
B9	$\geq 1.0 \times 10^9$	1.1×10^5	9.0×10^2	2.65×10^6	2.43×10^7	ND	1.01×10^6
B10	$\geq 1.0 \times 10^9$	ND	$\geq 1.0 \times 10^6$	2.81×10^6	$\geq 1.0 \times 10^9$	$\geq 1.0 \times 10^9$	ND
B11	$\geq 1.0 \times 10^9$	3.22×10^4	6.6×10^2	1.76×10^5	2.68×10^7	2.68×10^7	ND
B12	1.38×10^7	7.05×10^3	ND	2.0×10^4	1.01×10^7	1.01×10^7	ND
B13	$\geq 1.0 \times 10^9$	$\geq 1.0 \times 10^6$	$\geq 1.0 \times 10^6$	1.25×10^4	$\geq 1.0 \times 10^9$	$\geq 1.0 \times 10^9$	ND
B14	8.7×10^7	1.56×10^5	ND	ND	8.8×10^7	8.8×10^7	ND
B15	1.27×10^7	9.05×10^4	7.3×10^2	ND	2.01×10^7	2.1×10^7	ND
Minimum	2.0×10^6	ND	ND	ND	ND	ND	ND
Maximum	$\geq 1.0 \times 10^9$	$\geq 1.0 \times 10^6$	$\geq 1.0 \times 10^6$	1.0×10^7	$\geq 1.0 \times 10^9$	$\geq 1.0 \times 10^9$	3.3×10^7
Mean	3.63×10^8	4.94×10^5	1.44×10^5	1.77×10^6	1.54×10^8	2.07×10^8	2.43×10^6
SD	$\pm 4.13 \times 10^8$	$\pm 4.75 \times 10^5$	$\pm 3.37 \times 10^5$	$\pm 3.35 \times 10^6$	$\pm 3.44 \times 10^8$	$\pm 3.38 \times 10^8$	$\pm 8.47 \times 10^6$

Table 2: Microorganisms levels of the cheese samples (in Bayramiç) (cfu/g).

Sample No	TMAB	Yeast and Moulds	Coliform	*Staphylococci spp.*	*Lactobacillus spp.*	*Lactococcus spp.*	*Enterococcus spp.*
E1	1.37×10^8	ND	ND	3.08×10^4	ND	3.2910^8	1.0×10^3
E2	4.25×10^7	2.5×10^3	ND	2.67×10^4	ND	ND	6.1×10^6
E3	1.92×10^8	$\geq 1.0 \times 10^6$	2.85×10^4	ND	3.6×10^7	7.0×10^7	ND

E4	6.3×10^7	1.48×10^4	8.0×10^5	ND	1.2×10^7	5.4×10^7	7.0×10^5
E5	6.0×10^7	7.2×10^4	1.0×10^5	3.28×10^5	1.0×10^7	4.73×10^7	ND
E6	1.0×10^4	ND	ND	8.0×10^4	3.0×10^4	ND	ND
E7	2.99×10^8	$\geq 1.0 \times 10^6$	ND	3.9×10^5	1.17×10^8	1.03×10^8	ND
E8	$\geq 1.0 \times 10^9$	2.61×10^4	1.0×10^5	1.0×10^4	1.0×10^9	1.0×10^9	ND
E9	1.79×10^8	6.5×10^4	ND	2.65×10^6	6.85×10^6	6.78×10^7	ND
Minimum	1.0×10^4	ND	ND	ND	ND	ND	ND
Maximum	$\geq 1.0 \times 10^9$	$\geq 1.0 \times 10^6$	8.0×10^5	2.65×10^6	1.0×10^9	1.0×10^9	6.1×10^6
Mean	2.19×10^8	2.42×10^5	1.04×10^5	4.36×10^5	1.31×10^8	1.86×10^8	1.46×10^5
SD	$\pm 3.07 \times 10^8$	$\pm 4.30 \times 10^5$	$\pm 2.61 \times 10^5$	$\pm 8.60 \times 10^5$	$\pm 3.47 \times 10^8$	$\pm 3.21 \times 10^8$	$\pm 2.02 \times 10^6$

Table 3: Microorganisms levels of the cheese samples (in Ezine) (cfu/g).

Sample No	TMAB	Yeast and Moulds	Coliform	*Staphylococcus spp.*	*Lactobacillus spp.*	*Lactococcus spp.*	*Enterococcus spp.*
K1	2.0×10^6	5.40×10^3	ND	1.33×10^4	5.95×10^7	1.80×10^7	ND
K2	2.70×10^7	$\geq 1.0 \times 10^6$	ND	3.94×10^4	1.80×10^7	ND	5.50×10^5
K3	1.15×10^7	9.40×10^3	ND	1.80×10^3	1.10×10^7	ND	ND
K4	1.37×10^8	1.10×10^3	ND	1.99×10^4	3.60×10^7	1.18×10^7	1.00×10^6
K5	1.36×10^8	1.00×10^2	ND	2.70×10^3	ND	1.49×10^8	1.20×10^5
K6	2.20×10^7	5.20×10^3	ND	ND	1.15×10^6	2.05×10^6	ND
K7	5.50×10^8	2.60×10^3	ND	4.00×10^3	6.00×10^5	5.65×10^7	ND
K8	2.10×10^6	4.20×10^3	ND	ND	ND	2.40×10^7	ND
K9	$\geq 1.0 \times 10^9$	2.51×10^4	8.00×10^2	ND	3.55×10^6	2.57×10^6	5.03×10^4
K10	5.15×10^6	ND	ND	1.50×10^4	3.30×10^6	3.0×10^6	ND
K11	1.50×10^7	1.87×10^5	ND	ND	1.36×10^7	2.00×10^5	ND
K12	2.20×10^7	1.01×10^4	ND	1.0×10^3	6.30×10^7	2.01×10^7	ND
K13	1.28×10^8	2.91×10^4	ND	1.80×10^4	1.15×10^6	5.30×10^7	ND
K14	1.15×10^8	$\geq 1.0 \times 10^6$	ND	1.0×10^3	$\geq 1.0 \times 10^9$	$\geq 1.0 \times 10^9$	ND
Minimum	2.0×10^6	ND	ND	ND	ND	ND	ND
Maximum	$\geq 1.0 \times 10^9$	$\geq 1.0 \times 10^6$	8.00×10^2	3.94×10^4	$\geq 1.0 \times 10^9$	$\geq 1.0 \times 10^9$	1.00×10^6
Mean	1.55×10^8	1.63×10^5	5.71×10^1	8.29×10^3	8.24×10^7	9.57×10^7	1.23×10^5
SD	$\pm 2.82 \times 10^8$	$\pm 3.58 \times 10^5$	$\pm 2.41 \times 10^2$	$\pm 1.16 \times 10^4$	$\pm 2.65 \times 10^8$	$\pm 2.63 \times 10^8$	$\pm 2.92 \times 10^5$

Table 4: Microorganisms levels of the cheese samples (in Kepez) (cfu/g).

The frequency of population distribution of the microorganism groups are shown in Table 5. The average colony counts from BP Agar were evaluated as 1.77×10^6 cfu/g (ND- $\geq 1.00 \times 10^7$ cfu/g), 4.36×10^5 cfu/g (ND- $\geq 2.65 \times 10^6$ cfu/g), and 8.29×10^3 cfu/g (ND- $\geq 3.94 \times 10^4$ cfu/g) in cheese samples from Bayramiç, Ezine and Kepez, respectively. The dark grey to black, with/without clear zones colonies were counted together and data was given as *Staphylococcus spp.* All typical selected colonies (with/without clear zones) were identified as Gram (+), catalase (+), Oxidase (-) cocci. According to the coagulase test results coagulase positive *Staphylococcus aureus* was not detected.

The mean counts of *Lactococcus spp.* and *Enterococcus spp.* were detected as 2.07×10^8 cfu/g (ND- $\geq 1.00 \times 10^9$ cfu/g) and 2.43×10^6

cfu/g (ND- ≥ 3.30×10^7 cfu/g) in town Bayramiç, 1.86×10^8 cfu/g (ND-1.00×10^9 cfu/g) and 1.46×10^5 cfu/g (ND-6.1×10^6 cfu/g) in town Ezine, 9.57×10^7 cfu/g (ND- ≥ 1.00×10^9 cfu/g) and 1.23×10^5 cfu/g (ND-1.00×10^6 cfu/g) in town Kepez, respectively. The average colony counts from Rogosa Agar were evaluated as 1.54×10^8 cfu/g (ND- ≥ 1.00×10^9 cfu/g), 1.31×10^8 cfu/g (ND- ≥ 1.00×10^9 cfu/g), and 8.24×10^7 cfu/g (ND- ≥ 1.00×10^9 cfu/g) in cheese samples from Bayramiç, Ezine and Kepez, respectively. All typical colonies selected from M17 and KAA were identified as Gram (+), catalase (-), Oxidase (-) *cocci* and considered belong to genus *Lactococcus spp.* and *Enterococcus spp.*, respectively. The typical colonies selected from Rogosa Agar were identified as Gram (+), catalase (-), Oxidase (-) bacilli and considered belong to genus *Lactobacillus spp.*

Microorganisms	Percentage of samples in different population groups								
	ND	10^2	10^3	10^4	10^5	10^6	10^7	10^8	10^9
TMAB				2.63		10.52	36.84	34.21	15.78
Yeast and Moulds	13.15	2.63	21.05	26.31	7.89	28.94			
Coliform	65.78	10.52	2.63	2.63	13.15	5.26			
Staphylococcus spp.	21.05		13.15	39.47	13.15	7.89	5.26		
Lactobacillus spp.	15.78			2.63	2.63	18.42	47.36	2.63	10.52
Lactococcus spp.	13.15				2.63	13.15	42.10	18.42	10.52
Enterococcus spp.	65.78		2.63	5.26	10.52	13.15	2.63		

Table 5: Population distribution of the microorganisms of 38 home-made cheese samples (%).

TMAB were determined in all samples and total number counts of TMAB were differentiated between 10^4 and 10^9 cfu/g. Coliform was not determined in 25 of 38 (65.78%) cheese samples. Although *Staphylococcus spp.* were not found in 8 of 38 sample (21.05 %), in 30 of 38 samples the counts of *Staphylococci* were differentiated between 10^3 and 10^7 cfu/g. While the counts of *Lactobacillus spp.* (32 of 38 samples -84.19%) and *Lactococcus spp.* (5 of 38 samples -86.85%) were determinated high than 10^5 cfu/g, *Enterococcus spp.* was not found in 25 of 38 samples (65.78%) and the frequency of *Enterococcus spp.* counts were found between in 13 of 38 samples 10^3 and 10^7 cfu/g (Table 5).

Due to high water activity and biochemical composition, milk is an excellent medium for bacterial growth. As well as beneficial natural bacteria such as *lactobacilli*, *streptococci* and *lactococci*, pathogens can also grow in milk. This uncontrolled growth can lead to negative impacts on quality and safety of dairy products. Contamination of cheese with pathogens can launch out with their presence in the raw milk used for cheese making and their survival during process. Bacterial contamination can also go on during post-processing on account of improper sanitation and insufficient measures to prevent re-contamination [10]. There are several studies on cheeses scrutinizing the presence of microorganisms which are serious concern in terms of public health [6-10].

In a previous study which analyse 28 Civil cheese samples from Ankara in terms of presence TAMB, yeast and moulds, coliform bacteria, *E.coli* and *Staphylococci spp.* the counts of microorganisms have been detected as 1.10×10^8 cfu/g, 4.10×10^7 cfu/g, 1.10×10^3 EMS/g, 3.80×10^2 EMS/g and $<10^2$ cfu/g, respectively [11]. Aygun et al. have found the counts of TAMB 1.87×10^8 cfu/g, yeast and moulds 4.80×10^7 cfu/g, coliform bacteria 1.02×10^4 cfu/g, *E.coli* 4.27×10^3 cfu/g, and *Staphylococcus spp.* 2.51×10^3 cfu/g from Carra cheese which were purchased from different retail markets in Antakya [12]. While TAMB, yeast and youlds counts of our study have been similar, counts of coliform bacteria and *Staphylococcus spp.* have been higher than the results of these previous studies.

In a similar vein the study conducted with 40 çökelek samples from markets in Elazığ, the average counts of microorganisms have established for TAMB as 2.87×10^8 cfu/g, yeast and moulds as 3.10×10^7 cfu/g, coliform bacteria as 8.53×10^3 cfu/g, *Staphylococcus spp.* as 1.42×10^3 cfu/g, *Lactobacillus-Leuconostoc-Pediococcus* as 2.97×10^7 cfu/g and *Lactococcus spp.* as 2.97×10^7 cfu/g [1]. As far as another study on Gravier cheeses, the counts of TAMB, yeast and moulds, coliform bacteria, *Staphylococcus spp.*, *E.coli*, lactic acid bacteria and *Lactococcus spp.* have been detected as 2.30×10^7 cfu/g, 3.90×10^4 cfu/g, 1.00×10^3 cfu/g, 4.80 × 104 cfu/g, $<1.00 \times 10^2$ cfu/g, 5.10×10^5 cfu/g and 8.50×10^6 cfu/g, respectively [2]. Whereas results of TAMB, yeast and moulds in our study have been found similar, counts of coliform bacteria and *Staphylococcus spp.* have been higher than the results reported by Kamber et al. [2] Öksüztepe et al. [13]. Furthermore higher counts of lactic acid bacteria and *Lactococcus spp.* have been also detected in our study.

According to results of a study on 100 home-made white pickled cheese samples from street markets in Burdur it has been determinated that the frequency of microorganism counts ranged between as TAMB 4.60×10^6-1.00×10^9 cfu/g, yeast and moulds 10^4-10^5 cfu/g, coliform bacteria 10^2-10^5 cfu/g, *Staphylococcus spp.* 10^3-10^6 cfu/g, and *Enterococcus spp.*>10^3 cfu/g [4]. In a previous study on 105 Örgü Cheese samples without packaging which were sampled from several markets in Diyarbakır the contamination rates of yeast, moulds, coliform bacteria, *E.coli* 0157:H7, *Staphylococcus-Micrococcus spp.* were found 93.33%, 32.38%, 80.00%, 7.62%, and 84.76% respectively [14]. The percentage of contamination rates in this study have been found higher than our study's rates and *E.coli* 0157:H7 also have not been detected in our study. When the counts of microorganisms from each towns compared, TMAB have been found in all samples at high levels but, counts of coliform bacteria and *Staphylococcus spp.* in samples from Kepez have been found relative lower than those of Ezine and Byramic (P<0,05) (Table 6).

Microorganisms	Percentage of each towns in different population groups									
	Town	n	ND	10^2	10^3	10^4	10^5	10^6	10^7	10^8	10^9
TMAB	Bayramiç	15						6.66	40	26.66	26.66
	Ezine	9				11.11			33.33	44.44	11.11
	Kepez	14						21.42	35.71	35.71	7.14
Yeast and Moulds	Bayramiç	15	13.33		6.66	20	13.33	46.66			
	Ezine	9	22.22		11.11	44.44		22.22			
	Kepez	14	7.14	7.14	42.85	21.42	7.14	14.28			
Coliform	Bayramiç	15	46.66	20	6.66		13.33	13.33			
	Ezine	9	55.55			11.11	33.33				
	Kepez	14	92.85	7.14							
Staphylococci spp.	Bayramiç	15	13.33			40	20	13.33	13.33		
	Ezine	9	22.22			44.44	22.22	11.11			
	Kepez	14	28.57		35.71	35.71					
Lactobacillus spp.	Bayramiç	15	13.33					13.33	60		13.33
	Ezine	9	22.11			11.11		11.11	33.33	11.11	11.11
	Kepez	14	14.28				7.14	28.57	42.85		7.14
Lactococcus spp.	Bayramiç	15	6.66					13.33	40	26.66	13.33
	Ezine	9	22.22						44.44	22.22	11.11
	Kepez	14	14.28				7.14	21.42	42.85	7.14	7.14
Enterococcus spp.	Bayramiç	15	60			6.66	6.66	20	6.66		
	Ezine	9	66.66		11.11		11.11	11.11			
	Kepez	14	71.42			7.14	14.28	7.14			

Table 6: Population distribution of the microorganisms of each town (%).

These lower levels of microorganism counts from Kepez can be thought result of relatively good storage and retail conditions and more controlled by officers than Ezine and Bayramiç. According to the results from this study thirty eight cheese samples from bazaars of Bayramiç, Ezine and Kepez have not been consonant with acceptability levels fixed by Turkish Food Codex regulations [15]. These results indicate poor husbandry and hygiene practices during every step in production processes and/or unsuitable storage and retail conditions.

Food contamination, either from microbiological or chemical, is the highest concern for public health. Knowing main risks and contaminants in each food processing steps is crucial for the control them and prevent or diminish them from the food [16]. For producing more safe and quality foods adoption of risk assessment models such as Good Manufacturing Practices and HACCP are essential. Testing animal health, controlling milk quality, processing hygiene requirements, monitoring of critical points such as temperature during process, transport even retail sale are necessity for all food productions including cheese [17]. Inspection of food manufacturing compliance with the national/international regulation especially in home and/or small plants made foods exposed for sale in street markets or bazaars is also important for preventing public health. The results of present study also support these concerns.

Faecal pathogens such as *E. coli*, *Salmonella spp.*, *Listeria spp.* and *Vibrio cholerae* from irrigation water and contaminate agricultural products and food-producing animals are often the primary source of pathogenic agents responsible from human infections. Cross-contamination of pathogens from livestock farms, production plants and even retail environments to ready to eat or fresh foods, cheeses cause of either foodborne disease outbreaks or spoilage of food products which makes a big economic loss during storage [18].

Quick and safe detection of foodborne pathogens in every steps of food chain is a crucial issue for the food industry. Traditional microbiological methods which often take several days have supplied for the identification of bacterial strains by standard cell culture,

plating and biochemical tests. However, cultural methods may not be achieve to detect to some foodborne pathogens which might be in viable but non-culturable due to response to stress such as adverse nutrient, temperature or osmotic, oxygen or light conditions [19]. To overcome the drawbacks of traditional culture methods, alternative methods have been developed in the recent years which are mainly based on molecular approaches or protein based methods including ELISA, 2D-PAGE and mass spectrometry. For the development of more sensitive, multiplexed, faster systems for detection of foodborne pathogens, combinations of nanotechnology with the specific detection of DNA or protein are recent and innovative approaches. The use of micro and nano fluidics with or without nanoparticles, the integration of nanomaterial-based sensors with food packaging systems are the examples of these fast and reliable analytical methods [19]. In order to rapid microorganism detection, ATP bioluminescence based methods also have shown great interest due to significantly shorten time to detection without losing reliability [20].

According to this study results, cheese samples produced without uncontrolled conditions such as in small dairy farms or home and sold in bazaars of Bayramiç, Ezine and Kepez (Çanakkale) have high microorganism loads and do not meet the microbiological criteria in Turkish Food Codex regulations. These results emphasize the need to apply and maintain good hygiene practices and also suggest that more stringent checks should be carried out throughout food chain including retail conditions.

Conflict of Interest

No conflict of interest associated with this work.

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