

THE MICROBIOLOGICAL QUALITY OF CERTAIN TYPES OF TRADITIONAL CHEESES PRODUCED IN DÂMBOVIȚA COUNTY

CALITATEA MICROBIOLOGICĂ A UNOR SORTIMENTE DE BRÂNZETURI TRADIȚIONALE OBȚINUTE ÎN JUDEȚUL DÂMBOVIȚA

A.R. TURBATU^{1),*)},
C. SAVU¹⁾

ABSTRACT | REZUMAT

The microbiological quality of traditional cheeses is influenced by pathogenic microorganisms that can appear in cheeses through milk or later by indirect contamination during the production process. Contamination with pathogenic bacteria occurs due to the artisanal way of obtaining it without the involvement of modern technology, the place and space where the technological process takes place and the unpasteurised milk used. This study focusses on the identification and isolation of coagulase-positive staphylococci, coliform bacteria and bacteria of the genus *Salmonella* spp., which are the main pathogens that contaminate traditional cheeses and produce food poisoning. The assortments analysed were telemea cheese, burduf cheese and urda; each assortment has multiple varieties. As a result of the testing, it was shown that the highest values of staphylococcal contamination were 3.4×10^5 cfu/g (burduf cheese), but the assortments recorded many values < 100.0 cfu/g. Contamination with coliform bacteria was reduced, the highest value being 600.0 cfu/g (fresh sheep cheese), with over 90% of the samples having < 10.0 cfu/g. Contamination with bacteria of the genus *Salmonella* spp. was not reported for the samples analysed. Based on the results achieved, the superior quality of the traditional cheeses obtained in the Moroieni area was found.

Keywords: traditional cheeses, microbiological quality, coagulase-positive staphylococci, coliform bacteria, *Salmonella* spp.

Calitatea microbiologică a brânzeturilor tradiționale este influențată de microorganismele patogene care pot apărea în brânzeturi prin intermediul laptelui sau ulterior prin contaminare indirectă în timpul procesului de producție. Contaminarea cu bacterii patogene apare datorită: modului artizanal de obținere fără implicarea tehnologiei moderne, a locului și spațiului unde are loc procesul tehnologic și a laptelui nepasteurizat folosit. Acest studiu se concentrează pe identificarea și izolarea stafilococilor coagulazo-pozitivi, bacteriilor coliforme și a bacteriilor genului *Salmonella* spp., care sunt principalii agenți patogeni care contaminează brânzeturile tradiționale și produc toxiiinfecții alimentare. Sortimentele analizate au fost: brânza telemea, brânza de burduf și urda; fiecare sortiment având varietăți multiple. În urma testării s-a demonstrat faptul că valorile cele mai mari ale contaminării cu stafilococi au fost de 3.4×10^5 ufc/g (brânza de burduf), dar sortimentele au înregistrat multe valori < 100.0 ufc/g. Contaminarea cu bacterii coliforme a fost redusă, valoarea cea mai mare fiind de 600.0 ufc/g (telemea proaspătă de oaie), cu peste 90% din probe având < 10.0 ufc/g. Contaminarea cu bacterii ale genului *Salmonella* spp. nu a fost raportată pentru probele analizate. În urma rezultatelor obținute s-a constatat calitatea superioară a brânzeturilor tradiționale obținute în zona Moroieni.

Cuvinte cheie: brânzeturi tradiționale, calitate microbiologică, stafilococi coagulazo-pozitivi, bacterii coliforme, *Salmonella* spp.

Cheese is a dairy product, recognised as a nutritious food with a high content of proteins and minerals, especially calcium. Cheese is frequently eaten due to its benefits on human health, as well as due to its sensory and nutritional properties (2, 11, 17).

In Romania, cheese is produced both by traditional methods (handling is done manually and the technological process is not industrialised), and also by industrial methods (which are based on mechanisation). The production and consumption of artisanal or traditional cheeses, worldwide, are continuous increase. This study focuses on the collection and analysis of samples of traditional cheeses from the Moroieni area, Dâmbovița County. The Moroieni area is an area with

alpine pastures where animals are maintained during the summer season. The vast expanses of alpine pastures, which provide the supply of nutrient-rich green mass, ensuring a milk with superior properties, are preferred by shepherds who practice transhumance.

The traditional method of obtaining cheeses has been preserved for many years in the Moroieni area, and the entire craft is passed down from generation to generation. This includes several stages, specific to each individual assortment, the final product having a distinct flavour due to the maturation procedures (14, 15). Even if the stages of the technological process are strictly observed and the environment provides good sterility, due to the thick layer of snow and cold that persists for several months, the appearance of a number of potentially pathogenic microorganisms is possible. (10) states that since traditional cheeses are produced in small-scale production facilities, where pro-

1) University of Agronomic Sciences and Veterinary Medicine, Faculty of Veterinary Medicine, Bucharest, Romania

*) Corresponding author: turbatuandrei96@gmail.com

cess control is often difficult due to a lack of automation and control of environmental parameters and processing variables, there is a potential risk of microbial contamination and proliferation. Pathogenic bacteria can accidentally occur in the milk raw material or in the final product (3, 4).

Pathogenic bacteria multiply on favourable substrates, especially if the temperatures of the technological process are not observed. The growth of bacteria is also controlled by the predominant flora of lactic acid bacilli (5). There are many pathogenic bacteria that can contaminate cheeses, but four pathogens, including *Listeria monocytogenes*, *Salmonella* spp., *Staphylococcus aureus*, and *Escherichia coli*, are the predominant microorganisms that have caused outbreaks of infection due to contamination of traditional cheeses (12).

Salmonella spp. are Gram-negative bacteria, are shaped like bacilli and are facultatively anaerobic. They are part of the *Enterobacteriaceae* family, the most common being *Salmonella enterica* and *Salmonella bongori*. *Salmonella enterica* is the most well-known species with the following common serotypes: *S. Typhi*, *S. Paratyphi*, *S. Typhimurium*, and *S. enteritidis*. The serotypes *S. Typhimurium* and *S. Enteritidis* are frequently associated with cheese contamination (1).

Coagulase-positive *Staphylococcus aureus* is a facultatively anaerobic, Gram-positive bacterium that is ubiquitous in nature. Some strains produce pyrogenic, pepsin-resistant staphylococcal enterotoxins, which are potent emetic agents and cause staphylococcal food poisoning (7).

Escherichia coli has a bacillary, gram-negative and facultative anaerobic form, and is physiologically present in the intestinal tract of warm-blooded animals and humans. It is excreted into the external environment with faeces, which can lead to contamination of raw milk as a result of poor hygiene practices during milking (8).

The purpose of this study was the microbiological analysis of traditional cheeses obtained in Dâmbovită County. The assortments analysed were telemea cheese, burduf cheese and urda; varieties characteristic of this area that included several varieties, especially due to the origin of the milk.

MATERIALS AND METHODS

The materials used in this study were obtained from local producers, as well as from sales markets, thus having access to a wide variety of products. It is worth mentioning that the origin of milk is not always known. The samples were collected from batches of varying sizes, depending on the cheese assortment analysed. The batches ranged from about 10 kg to larger batches of about 250 kg.

The samples were collected in Dâmbovită County, Moroieni commune, a region famous for its traditions in cheese production. This selection of the study area was supported by its local reputation for the quality

and diversity of traditional products. The sample collection process started in January 2024 and was completed in November of the same year, thus ensuring relevant coverage of seasonal diversity and possible fluctuations in milk composition throughout the seasons. The samples collected consisted of the selection of the following assortments:

Telemea cheese – comprising a total of 30 samples analysed; this assortment presents the following varieties: cow's milk cheese (10 samples collected from batches between 50-100 kg), sheep's milk cheese (6 samples collected from batches between 8-30 kg), old cow's milk cheese (5 samples collected from batches between 83-150 kg), old sheep's milk cheese (2 samples collected from batches of 20 and 47 kg), fresh cheese from cow's milk (5 samples collected from batches 14-20 kg), and fresh sheep's milk cheese (3 samples collected from batches of 19, 23, and 27 kg).

Burduf cheese –comprising a total of 25 samples analysed and harvested from batches between 20-250 kg.

Urda – comprising a total of 14 samples analysed; this assortment presents the following varieties: sweet urda (3 samples collected from batches of 14, 22, and 26kg), salted urda (5 samples collected from batches 18-34 kg), cow's milk urda (4 samples collected from parrot batches 12-30 kg), sheep's milk urda (2 samples collected from batches of 13 and 15 kg).

The methods used were those characteristic and approved for the identification and isolation of each type of bacteria. These methods are found in Commission Regulation (EC) No 2074/2005 (3), where there is information on collection, analysis and interpretation. As mentioned before, the categories of bacteria for which the cheeses were tested are the following: coagulase-positive staphylococci, coliform bacteria and bacteria of the genus *Salmonella* spp.

The method used for the identification and isolation of coagulase-positive staphylococci was in: SR EN 6888/2005 (19), this is a European standard adopted by the European Organization for Standardization (CEN), being adopted in Romania, under the SR (Romanian Standard) logo is applied in the field of quality control and food safety. SR EN 6888/2005 is entitled "Microbiological methods. Determination of the number of coagulase-positive *Staphylococcus aureus* and *Staphylococcus* spp."

The method used for the identification and isolation of coliform bacteria was: SR EN 16649-2/2007 (20) entitled 'Microbiology of food and animal feeding stuffs – Horizontal method for the enumeration of beta-glucuronidase-positive *Escherichia coli*', is part of the series of European standards relating to methods of microbiological analysis of food and feed. This standard focuses specifically on the detection and counting of *Escherichia coli* bacteria and coliform bacteria, which are important indicators of food hygiene and safety.

The method used for the identification and isolation of bacteria of the genus *Salmonella* spp. was: ISO 6579/2002 (18) entitled "Microbiology of food and animal feeding stuffs – Horizontal method for the detection of *Salmonella* spp", which is an international stan-

dard defining methods for the isolation and identification of *Salmonella* bacteria in food and food intended for human and animal consumption. Its main purpose is to provide a standardized and reliable approach for the detection of Salmonella in food and food.

RESULTS AND DISCUSSION

As can be seen in the following table (Table 1), the varieties of telemea cheese analysed were multiple, and the number of samples was large enough to demonstrate the superior quality of traditional cheeses. Coagulase-positive staphylococcal contamination recorded the maximum value (2×10^5 cfu/g) within the cow's cheese variety, making the sample non-compliant. High values were also recorded in the sheep's cheese variety (4.1×10^3 cfu/g) and fresh cow's cheese (1.1×10^3 cfu/g). The very low values recorded were within the variety cow cheese (<10.0 cfu/g), sheep cheese (<10.0 cfu/g) and fresh sheep cheese (44.0 cfu/g). These results demonstrate that the telemea cheese is of high quality, with minimal contamination with coagulase-positive staphylococci. In a study conducted by (14), low values of the degree of contamination were also recorded in Braşov county, where most samples recorded values <10.0 cfu/g. Another study (16) reveals the very low values obtained by telemea cheese, where all the analysed assortments recorded values <10.0 cfu/g. During the contamination with coliform bacteria, 3 samples were non-compliant, namely: a cow's cheese sample (544.0 cfu/g), a sheep's cheese sample (465.0 cfu/g) and a fresh sheep's cheese sample (600.0 cfu/g). The rest of the samples recorded values <10.0 cfu/g. The results may be comparable to the study conducted by (16), where the values were below 10.0 cfu/g. Another study conducted by (9) states that telemea cheese obtained from unpasteurised milk recorded values higher than 100, reaching up to 1000 cfu/g. All samples analysed were negative for Salmonella contamination, a result also obtained by (14).

Table 1
Microbiological results of telemea cheese

Assortment	No. sample	The microbiological parameters investigated	
		Coagulase-positive staphylococci (cfu/g*)	Coliform bacteria (cfu/g)
Cow's milk telemea	10	88.0- 2×10^5	<10.0 -544.0
Sheep's milk telemea	6	<10.0 - 4.1×10^3	<10.0 -465.0
Aged cow's milk telemea	5	138.0- 8.3×10^2	<10.0
Aged sheep's milk telemea	2	835.0-876.0	<10.0
Fresh cow's milk telemea	5	253.0- 1.1×10^3	<10.0
Fresh sheep's milk telemea	3	44.0-352.0	<10.0 600.0

cfu/g - colony-forming units per gram

In the following table (Table 2) you can see the degree of contamination of the urda, with the 4 varieties analysed. The values of coagulase-positive staphylococcus contamination were between 14.0 cfu/g (sweet urda) and 785.0 cfu/g (cow's milk urda). Contamination with coliform bacteria showed minimum values, respectively <10.0 cfu/g for all varieties. The samples were negative for contamination with *Salmonella* spp. (13) states that all samples analysed by urda showed values lower than 10 cfu/g for contamination with coliform bacteria.

Table 2
The level of contamination with pathogenic bacteria in urda cheese

Assortment	No. sample	The microbiological parameters investigated	
		Coagulase-positive staphylococci (cfu/g*)	Coliform bacteria (cfu/g)
Sweet urda cheese	3	14.0-387.0	<10.0
Salted urda cheese	5	20.0-100.0	<10.0
Cow's milk urda cheese	4	<100.0 -785.0	<10.0
Sheep's milk urda cheese	2	<100.0 -102.0	<10.0

*cfu/g - colony-forming units per gram

The last table (Table 3) reveals the degree of contamination of bellows cheese. As can be seen, the microbial load is not directly proportional to the size of the batches. The highest value is presented by sample 19 (3.4×10^5 cfu/g), which is harvested from a batch of 130 kg, making the batch non-compliant. Sample 7 (9.5×10^4 cfu/g) showed a high microbial load, although the batch was 26 kg. Values <10.0 cfu/g were also recorded in small batches. (14) showed that the microbial load with staphylococci was significantly higher in the case of large batches. The contamination with coliform bacteria was considerable in the case of sample 22 (456.0 cfu/g), where the analysed batch had 40 kg; the rest of the values were below 78.0 cfu/g, many reaching up to <10.0 cfu/g. (6) states that following the analysis of the cheeses from Transylvania, the number of coliform bacteria was very high, with values up to 8×10^4 cfu/g., making a very large number of non-compliant samples. In another study conducted by (14), the values recorded were <10.0 cfu/g. For the analysed samples, a negative result was obtained for contamination with *Salmonella* spp.

CONCLUSIONS

The collection and analysis of the samples were carried out over a period of 11 months, starting in January 2024 and ending in November 2024. During this period, a total of 69 samples were collected and analysed. The traditional cheese varieties analysed were: telemea cheese, burduf cheese and urda. Within

Table 3
The microbiological contamination of burduf cheese from the Moroieni area

Sample number	Quantity	The microbiological parameters investigated		
		Coagulase-positive staphylococci (cfu/g)	Coliform bacteria (cfu/g)	<i>Salmonella</i> spp. (cfu/g)
1	20	588.0	<10.0	0
2	77	267.0	<10.0	0
3	83	760.0	<10.0	-
4	193	9.6x10 ²	<10.0	0
5	45	5.9x10 ²	<10.0	0
6	76	2.4x10 ³	<10.0	0
7	26	9.5x10 ⁴	<10.0	0
8	152	3.3x10 ²	35.0	-
9	40	134.0	<10.0	0
10	65	397.0	<10.0	0
11	94	9.5x10 ³	<10.0	-
12	100	250.0	78.0	0
13	56	<100.0	<10.0	0
14	210	7.1x10 ²	<10.0	0
15	250	5.9x10 ³	<10.0	0
16	20	<100.0	<10.0	-
17	20	33.0	<10.0	0
18	76	79.0	<10.0	0
19	130	3.4x10 ⁵	<10.0	0
20	45	321.0	<10.0	0
21	68	<10.0	<10.0	-
22	40	699.0	456.0	0
23	95	799.0	<10.0	0
24	185	9.9x10 ³	<10.0	0
25	25	<10.0	<10.0	0

cfu/g - colony-forming units per gram

these assortments, several varieties are distinguished, mainly according to the origin of the raw material. The study was carried out in Dâmbovița County, within the Moroieni commune, with the aim of establishing the degree of contamination of traditional cheeses with pathogenic bacteria. The microbiological quality was established following the isolation and identification of pathogenic bacteria such as: coagulase-positive staphylococci, coliform bacteria and bacteria of the genus *Salmonella* spp.

In case of coagulase-positive staphylococcus contamination, the analysed cheeses obtained low values. Telemea cheese with cow's cheese and fresh sheep's cheese showed the lowest values of 88.0 cfu/g and 44.0 cfu/g, respectively. Sweet urda showed the value of 14.0 cfu/g, and the lowest values were reached by burduf cheese, namely <10.0 cfu/g. Higher values were also observed in sheep cheese 4.1x10³ cfu/g, and the highest value was recorded in bellows cheese 3.4x10⁵ cfu/g, being the only non-compliant sample.

The degree of contamination with coliform bacteria has been significantly reduced. The highest value in telemea cheese was 600.0 cfu/g. Urda showed a very low degree of contamination <10.0 cfu/g for all varieties, and burduf cheese showed a sample with a value of 456.0 cfu/g. Within this contamination, a total of 4 samples were non-compliant. In case of contamination with bacteria of the genus *Salmonella* spp. All the samples analysed were negative. The traditional

cheeses analyzed showed a low degree of contamination, emphasizing the high standards of food safety in the technological process, which proves the superior quality of the traditional products obtained in the Moroieni area. These results confirm that the traditional methods used and proper hygiene can provide safe products for consumption.

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