Comparative analysis of hygienic quality of cottage cheese from Sarajevo and Zagreb markets during summer and winter seasons

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ABSTRACT

Objectives: Traditional, homemade fresh cheese is available for sale in local city markets throughout the Balkan region. Aim of the study was to examine the hygienic properties of the aforementioned fresh cheese, which is sold in numerous city markets of two European capital cities during the two seasons.

Materials and methods: This research project analysed 60 samples of cheese, taken from markets in Sarajevo and Zagreb, during the summer and winter seasons of 2015 and 2016. All cheese samples were microbiologically processed using ISO methods, and the results were interpreted according to the microbiological criteria for food.

Results: In all the collected samples of cheese in both cities, it was established that there was no presence of Salmonella spp. and Listeria monocytogenes. Due to the specific presence of bacterium Escherichia coli in eight samples of cheese (n=30) in the market places in Zagreb (26.6%), and coagulase-positive staphylococci (Staphylococcus aureus) in two samples of cheese (n=30) sampled from the market in Sarajevo (6.6%), out of all of the samples of cheese (n=60), ten samples, or 16.66% were hygienically incorrect. The total number of hygienically improper samples of cheese, because of the findings of S. aureus in numbers greater than the maximum permitted in relation to the time of year, was higher in winter than in summer.

Conclusion: There is a need to improve hygienic conditions in the entire cheese manufacturing process. People who consume fresh cheese on a daily basis are exposed to a potential risk of developing food-borne infections and intoxications, due to the microbiologically poor quality of cheese. Competent authorities should carry out supervision, and control production, while manufacturers themselves should implement a system of self-control that is subject to inspection and regulation.

Keywords: fresh cow cheese, ISO methods, microbiological criteria, E. coli, S. aureus

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Introduction

Cheese is a food that is not subject to heat treatment and is consumed fresh, as a finished product. Fresh cheese is prepared from coagulated milk, from which cream separates the top layer, while soft curd is drained in a cheese mass which is immediately in a fresh condition that is suitable for consumption. Because of this, cheese can be very dangerous to a human’s health, as it may cause food-borne infections and intoxications, therefore posing a significant public health concern. Fresh cheese is produced domestically based on the established recipe. A large number of consumers prefer “domestic” dairy products, assuming that the quality is better than that which is produced in facilities of the more modern dairy industry. (1) Cheese is made up in a high percentage of water, low milk fat content and high acidity. It is distinguished by a characteristic taste, smell, color and consistency. In the production of cheese, pasteurised or raw milk may be utilised from completely healthy cows, which must meet prescribed requirements in terms of physical, chemical and microbiological quality. (2) Cheese production is fairly simple. Freshly milked milk at about 25°C (room temperature) is fermented during 24-48 hours. During this time, the naturally occurring lactic acid bacteria produce acid curd which is cut to the size of a match box, and drained into appropriate molds, or it may be reheated to a temperature between 40 and 50°C in order to facilitate the outflow of whey therefrom. Separate sour cream is consumed together with cheese. (3) Previous studies of the composition, hygiene and quality conducted on samples of fresh cheese pointed to the great variation of their respective values. Most microorganisms that cause spoilage are very adaptable, and can very easily contaminate raw milk and dairy products. Thus, evaluation and control of the presence of these microorganisms, the cause of spoilage, is the only way to ensure the correctness of fresh cheese.
Raw milk must be of standard quality according to the number of somatic cells. These are epithelial cells of the udder and blood cells (granulocytes, lymphocytes, leukocytes, etc.), and their higher numbers refer to udder inflammation (mastitis). The milk of healthy animals contains generally less than 200,000 and often less than 100,000 somatic cells/mL. In addition, milk must have a standard quality when there are microorganisms present therein. According to EU legislation, the maximum permitted number of live microorganisms – CFU (Colony Forming Units) in a mL of raw milk may be no higher than 100,000 10^5cfu/mL. (4, 6, 7, 8) The demand for high standards of milk quality imposed by consumers requires a need to minimise the microbiological contamination of milk. This can be achieved by milking in hygienic conditions, and the milk being treated in an adequate technological and hygienic manner after milking. (9) Freshly milked milk is warm and has a pH of 6.5-6.7 which is a favourable environment for the growth of most microorganisms. When the milk is cooled, it can be a suitable medium for the development of some bacteria, especially if kept cool for a long time, which is advantageous for the development of psychotropic bacteria, which optimally grow at 20-30°C, but quickly adapt to lower temperatures. (10) Even with the best preventive measures, especially in terms of hygiene, it is not possible to get raw milk without any microorganisms in it. They directly reach the udder through the mammary gland openings, reproduce in the mammary gland, and thus become a part of the regular microflora of raw milk. Some bacteria are killed because of the bactericidal activity of tissues, while others are resistant and survive. After the performed milking hygiene, raw milk typically contains less than 5,000 organisms/mL. However, microorganisms come from the immediate environment in milk, and their number may rise up to several hundred thousand/mL. (10) Freshly milked milk contains microflora from the inside of a healthy udder, which is also called the primary microflora of milk. It consists of bacteria of the genus Micrococcus (60-90%) so they are called udder micrococci. There are also some bacteria of the genus Streptococcus. From the epidermis of the udder, the bacteria Streptococcus epidermis may appear in the milk. Corynebacterium may also be present, e.g. Corynebacterium bovis. (10) The first stream of milk usually contain micrococci and Corynebacterium bovis. (11) In freshly milked milk, there can also be found the bacteria that cause mastitis, which are usually bacteria from the genera Staphylococcus, Streptococcus, Escherichia and Corynebacterium pyogenes. If some microorganisms subsequently reach the milk, they form the secondary microflora. These are mainly bacteria, and rarely yeasts and mold. After the aseptically collecting milk of healthy cows, raw milk usually contains several hundred bacteria in mL, often less than 100 CFU/mL. (10) Then, in the procedure of milk processing, there are great opportunities for its further contamination. Faeces of livestock is the biggest source of enteropathogenic bacteria. Milking equipment and also water, may both be the source of a thermostable micrococcus (Microbacterium lacticum), as well as many Gram-negative psychotropic bacteria belonging to the genera Pseudomonas, Aeromonas, Achromobacter, Alcaligenes, Flavobacterium. Also, water and milking equipment can be sources of coliform infections. Bacteria present in large numbers can impact major changes in raw milk (degradation of proteins and fats), changing the composition of milk that affects its technological properties and consequently on the quality of many dairy products, especially cheese. (10) Contamination of milk can come from bacteria from the air, but also due to irregular milking, and dirty milking equipment, as well as unhygienic containers for storing and transporting milk. (12) In most cases, microorganisms - the cause of milk and dairy product spoilage, are a harmless group of microorganisms. (13) However, their presence diminishes product quality. Cheese, like other products containing a large amount of protein, are a favourable medium for the development of pathogenic microorganisms. The most commonly isolated pathogenic species potentially present in cheese are Listeria monocytogenes, Staphylococcus aureus, Escherichia coli and Salmonella spp. (14) The quality of the cheese depends on the number and type of microorganisms present, however, no technological process can absolutely guarantee the complete safety of the product. (15) For pathogenic bacteria, pasteurisation of milk is the safest way to prevent possible infections, even more so because a significant number of cheese is produced from raw milk. In controlling the hygienic correctness of soft cheeses for the presence of S. aureus bacterium by standard methods, the establishment of living cells is justified 48-72 hours after production. After 72 hours, only the existence of staphylococcal endotoxin is monitored. The survival of L. monocytogenes in cheese is directly conditioned by the technological process of producing a particular type of cheese. The survival of E. coli is an indicator of faecal contamination during the production and packaging of cheese. A large number of coliform bacteria do not always signify the presence of pathogenic bacteria, but clearly indicates an improper production method. Faecal contamination of the skin and udder of animals permits salmonella to contaminate milk. (16) 

### Material and methods

In order to examine the hygienic correctness of fresh cow cheese, 60 cheese samples were analysed, 30 of which were produced in Sarajevo and 30 in Zagreb,
sold in city marketplaces. The study was conducted during two seasons (summer and winter), with 15 samples collected and analysed in the summer and 15 in the winter. Research on the presence or absence of pathogenic bacteria, the identification of their number (qualitative and quantitative ISO standard methods), was carried out according to existing microbiological criteria (the same in both countries as a part of EU legislation). For analysis, 200-500 grams of fresh cow cheese was taken, and all samples were adequately transported (portable refrigerator) and delivered to the laboratory as soon as possible. Samples of fresh cheese from Sarajevo markets were analysed in a laboratory accredited to ISO 17025 (ILKK - Federal Institute for Agriculture-Sarajevo). The samples of fresh cheese from the Zagreb market were analysed in a laboratory that was also accredited according to the ISO 17025 standard (Microbiological Laboratory - ZIN LAB Laboratory for testing food safety of foodstuffs and objects of general use - Veterinary Station of the City of Zagreb).

In examining the microbiological correctness of fresh cheese for sale throughout city markets, the samples were tested for the following microorganisms: *Escherichia coli*, *Listeria monocytogenes*, *Salmonella* spp. and coagulase positive staphylococci (*Staphylococcus aureus*). Bacteriological examination was carried out according to the procedures described in ISO methods. Methods of the International Organisation for Standardisation (ISO) are the most widespread official methods used in microbiological research, isolation and the determination of certain bacterial species.

**RESULTS**

Of the total number of samples of fresh cheese, 30 were sampled from markets of Sarajevo and 30 from the markets of Zagreb. Of the 30 samples, 15 were collected during the winter, and 15 during the summer, equally in both cities.

From Table 1, it is visible that the total *E. coli* count on average was $1.20 \pm 1.55 \log_{10} \text{cfu/g}$ in the summer, and slightly lower in the winter, amounting to $1.06 \pm 1.6 \log_{10} \text{cfu/g}$. Compared to the standard of hygienic malfunction of samples with more than 1,000 cfu/g *E. coli*, a large number of unsatisfactory samples (n = 5) were determined during winter, but not significant compared to the results of the microbiological examination of the summer cheese sampling ($p = 0.73$).

Table 2. shows that in the summer period, in samples of fresh cheese an average higher number of bacteria *S. aureus* ($1.91 \pm 1.39 \log_{10} \text{cfu/g}$) was determined in relation to winter sampling ($1.16 \pm 1.59$). *S. aureus* was present in more than the maximum permissible levels in the winter period in relation to the summer period with the threshold level of significance ($p = 0.058$).

Of the total number of samples (n = 60), 5 samples (8%) of fresh cheese were produced from pasteurised milk, while 55 samples (92%) were produced from unpasteurised milk. Figure 1 shows the ratio of samples compared to a criterion of heat treatment of milk for cheese production.

Results of bacteriological examination of samples of fresh cheese produced from pasteurised milk in relation to the determined number of *E. coli* and *S. aureus* bacteria and the results of the bacteriological examination of fresh cheese produced from unpasteurised milk are shown in Tables 3 and 4.

Table 3 shows that the average number of *E. coli* was higher in the samples of cheese from unpasteurised milk ($1.23 \pm 1.59 \log_{10} \text{cfu/g}$). In samples of cheese from pasteurized milk, *E. coli* has not been established.

The number of samples that are deemed unfit due to findings of *E. coli* in the number of > 1,000 cfu/g is not significantly higher ($p = 0.09$) in samples of unpasteurised milk compared to samples produced from pasteurised milk.

**Table 1. Statistical figures of the number of Escherichia coli in samples of fresh cheese in relation to the season of sampling**

<table>
<thead>
<tr>
<th>Indicator</th>
<th>Season</th>
<th>N</th>
<th>Average</th>
<th>Standard deviation</th>
</tr>
</thead>
<tbody>
<tr>
<td>E. coli, $\log_{10} \text{cfu/g}$</td>
<td>winter</td>
<td>30</td>
<td>1.06</td>
<td>1.6</td>
</tr>
<tr>
<td></td>
<td>summer</td>
<td>30</td>
<td>1.20</td>
<td>1.55</td>
</tr>
</tbody>
</table>

**Table 2. Statistical indicators of the number of Staphylococcus aureus in fresh cheese samples compared to the sampling season**

<table>
<thead>
<tr>
<th>Indicator</th>
<th>Season</th>
<th>N</th>
<th>Average</th>
<th>Standard deviation</th>
</tr>
</thead>
<tbody>
<tr>
<td>S. aureus, $\log_{10} \text{cfu/g}$</td>
<td>winter</td>
<td>30</td>
<td>1.16</td>
<td>1.59</td>
</tr>
<tr>
<td></td>
<td>summer</td>
<td>30</td>
<td>1.91</td>
<td>1.39</td>
</tr>
</tbody>
</table>

Figure 1. The relationship of fresh cheese samples with respect to the criterion of pasteurised milk.
From Table 4, it can be seen that the average value of *S. aureus* in the samples of fresh cheese produced from unpasteurised milk was $1.60 \pm 1.55 \log_{10} \text{cfu/g}$, while that number in pasteurised milk was $0.83 \pm 1.15 \log_{10} \text{cfu/g}$, and was less than $1 \log_{10}$ relative to the cheeses obtained from unpasteurised milk. The number of samples considered hygienically incorrect due to *S. aureus* findings $>100,000 \text{ cfu/g}$, is not significantly higher ($p = 0.28$) for cheese samples produced from unpasteurised milk compared to those produced from pasteurised milk.

Tables 5 and 6 show the results of linear and logistic regression analysis.

A linear regression analysis (Table 5) was used to evaluate the association of risk factors with *E. coli* and *S. aureus* bacteria in cheese samples. A statistically significant correlation was the place of cheese sold (city and market) with increased *E. coli* or *S. aureus*. A significantly higher number of *E. coli* was found in the samples from Zagreb ($\beta = 0.18; \text{ CI: 0.00; 1.57}; p = 0.05$) and a significantly smaller number of *S. aureus* compared to cheese samples from Sarajevo ($\beta = -0.30; \text{ CI: -0.99; 0.39}; p = 0.04$). Also, there is a marginally significant association between the seasons and the number of *S. aureus* bacteria. A large number of these microorganisms in fresh cheese were found in the summer than in the winter season ($\beta = 0.75, \text{ CI: -0.02, 1.52, p = 0.058}$).

A simple logistic regression conducted to assess the risk factor’s impact on the likelihood that cheese would contain an increased, unsatisfactory number of *E. coli* bacteria or *S. aureus*. As is evident according to Table 6, only the city model was statistically significant. This model shows the difference in the exceedingly high values of *E. coli* in the cheese that was sold in Zagreb and which was sold in Sarajevo. Fresh cheese produced in Zagreb had 8.52 times the number of *E. coli* than the cheese sampled in Sarajevo (OR=8.52, CI: 0.98; 74.39; $p=0.05$). Simple logistic regression was performed to assess the impact of risk factors likely to increased the unsatisfactory number of *E. coli* or *S. aureus* in cheese. The production of cheese using unpasteurised milk is twice as likely associated with the excessive value of the number of *E. coli* in compar-
son to the production of cheese using pasteurised milk (OR = 8.52, CI: 0.11; 39.83; p = 0.68). This difference was not statistically significant due to the insufficient number of samples (large confidence interval). Other variables did not show significant differences to the excessively high number of bacteria in the study, *E. coli* and *S. aureus*, and the number of samples with too high of *S. aureus* (one sample) is too small for this analysis.

**DISCUSSION**

All of the above data indicates the fact that the bacteria *S. aureus* and *E. coli* are frequent contaminants of fresh cheese. Their presence may indicate the lack of efficient hygiene in cheese production, and also potentially of milk-based procedures after milking. Likewise, as in the research of Samaržija et al. (2007), we can conclude that the use of raw milk does not necessarily increase the risk of contamination of the cheese by *S. aureus* in comparison with the use of pasteurised milk. It is important to note from the aspect of health safety of fresh cheeses that *L. monocytogenes* and *Salmonella spp.* have not been detected.

Statistics showed a significant relation of the city and marketplace of sale of cheese with the finding that shows an increased number of *E. coli* or *S. aureus*. There appeared to be a significantly higher number of *E. coli* identified in samples of cheese from Zagreb ($\beta = 0.18$, CI: 0.00, 1.57, p = 0.05) and a significantly smaller number of *S. aureus* compared to the samples of cheese Sarajevo ($\beta = -0.30$, CI: -0.99; 0.39, p = 0.04). There is also a marginally significant relationship between the season and the number of *S. aureus*. In the summer, a greater number of these microorganisms are evident in the fresh cheese than in the winter ($\beta = 0.75$, CI: -0.02, 1.52, p = 0.058), although one unsatisfactory sample was confirmed only in the winter sampling. Fresh cheese produced in Zagreb had 8.52 times of a greater value of the number of *E. coli* in relation to the cheese sampled in Sarajevo.

A deviation from the reference values, that is, those with more than $1 \times 10^3$ cfu/g *E. coli* was recorded more often in winter, and a greater number of unsatisfactory samples (n = 5) were found, during the summer cheese sampling, but was not significant compared to the results of the microbiological examination. Overall, the number of *E. coli* was approximately $1.20 \pm 1.55$ log$_{10}$ cfu/g in summer, and only slightly lower in winter $(1.06 \pm 1.6$ log$_{10}$ cfu / g).

From the results of the *S. aureus* findings, it is evident that their presence in Sarajevo was determined in two samples in a number higher than the allowed reference value, once in cheese produced from pasteurised milk and once in cheese produced from unpasteurised milk, while in Zagreb the number of bacteria did not exceed the permitted values (p = 0.04.). If we observe the number of hygienic defective samples in relation to seasons, we see that this number is higher in winter than in the summer with a significant level of significance. Rosengren et al. (2010) found that *S. aureus* was above $4 \log_{10}$ cfu/g in fresh and soft cheeses, while *E. coli* was detected in 34% of cheese from raw milk and 3% of cheese from pasteurised milk. The highest *E. coli* > $6.62 \log_{10}$ cfu/g, was detected in fresh raw milk cheese with the addition of starter cultures. *L. monocytogenes* were not detected in either sample of cheese. In fresh cheese from pasteurised milk, *S. aureus* was found in 12% of samples, and *E. coli* in 7% of samples.

In the summer season, samples of fresh cheese were found to have an average larger number of *S. aureus* $(1.91 \pm 1.39 \log_{10}$ cfu/g) compared to winter sampling $(1.16 \pm 1.59 \log_{10}$ cfu/g).

**CONCLUSIONS**

The education of producers is needed in order to improve the production of milk and fresh cheese, with emphasis on hygiene in primary production and processing.

At the Sarajevo market, officials should ensure that all sellers of dairy products, including cheese, have refrigeration cabinets during sale hours, as is the case in Zagreb markets, and do not allow the sale of highly perishable foods outdoors. In addition, there must be a strengthening of the supervision of the competent veterinary inspection in order to prevent alimentary infections and consumer intoxications.

Consumers who are increasingly informed about the food they consume, require new products on the market, that are minimally heat-treated, appealing to the senses, and harmless to their health.

**DECLARATION OF INTEREST**

The authors declare no conflict of interest.
REFERENCES