Pathogen presence in cottage cheese sold for public consumption at Sarajevo Canton markets

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Abstract

Cottage cheese is the largest segment of the dairy market and is most often consumed as a fresh food. The microbiological quality of domestic cottage cheese can pose a problem for public health. Cottage cheese belongs to a group of foods having a potential public health risk. The aim of this study was to conduct microbiological research to determine the level of sanitary safety of cottage cheese types acquired from the most frequents markets of Sarajevo Canton (Bosnia and Herzegovina). Of the total (*n*=40) analysed cheese types, 22 samples (55%) proved to be meet sanitary requirements, while 18 samples (45%) did not meet microbiological quality, pursuant to the microbiological criteria for cheese stipulated by the National Regulation of Microbiological Criteria B&H and Guidelines for Microbiological Food Criteria B&H. The study included 24 samples of pasteurised and 16 samples of unpasteurised milk. However, five cheese samples (20.8%) from pasteurised milk, and 13 samples (81.3%) from unpasteurised milk were non-compliant. Microbiological analysis was conducted for compulsory and several recommended microorganisms: *Salmonella* spp., *Listeria monocytogenes*, coagulase positive staphylococci and other species (*Staphylococcus aureus*), *Escherichia coli*, and yeasts and moulds. Our findings confirm that the most common bacteria were *S. aureus* and *E. coli*. Also, yeasts and moulds were detected but were within the permissible concentrations. *Salmonella* spp. and *L. monocytogenes* were not detected in any of the examined samples.

Key words: *cottage cheese; unpasteurized milk; pasteurized milk; Escherichia coli; Staphylococcus aureus; Sarajevo Canton markets*

Introduction

Milk and dairy products contain many components of high biological value (glycerides, lipids, proteins), and a wide range of mineral salts and vitamins, and therefore, milk and dairy products can be considered a complete

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food. Cottage cheeses hold an important place in the human diet due to their high nutritional value, and also because of the traditional heritage of this food (Quintanilla et al., 2019). Cottage cheese, due to its moisture and nutrient content, can be highly suitable for the growth of different microorganisms and pathogens (Abujnah et al., 2016). Cheese is one of the most complex, fascinating, and diverse foods enjoyed today (Almena-Aliste and Mietton, 2014).

Cottage cheese is not heat-treated and is consumed fresh as the final product. Different types of cheese contain certain microorganisms that are very important in the process of its production and maturation. Depending on the type of cheese, these microorganisms can be divided into primary or secondary microorganisms. The biochemical interaction between microorganisms and the composition of milk enzymes determines the taste and texture of cheese (Criste et al., 2020; Nam et al., 2021). The production of cheese from raw milk is also a significant public health problem, as it can be a source of various pathogens. Milk is a raw material that is suitable for the growth and development of many microorganisms such as enterobacteria, an indicator of hygiene failures during production, and also of yeast and moulds, and other pathogens such as *Staphylococcus aureus* and *Listeria monocytogenes*. Microbial species and their initial number, the presence of other microflora, the physiological state of pathogenic bacteria, pH, and the type and composition of cheese, will determine the ability of pathogens to survive in the cheese (Landeka et al., 2019). Of all the pathogenic bacterial species that may potentially be present in cheese, *Listeria monocytogenes*, *Staphylococcus aureus*, *Escherichia coli* and *Salmonella* spp. are the most commonly isolated species. To prevent the growth of these pathogenic bacteria, the pasteurisation of milk is the most effective measure. The health condition of dairy cows must be monitored, and hygienic measures implemented in the process of collecting milk and cheese production (Havranek et al., 2014). According to the EU legislation, the maximum permitted number of live microorganisms (CFU - colony forming units) in 1 mL of raw milk may not exceed 10^5 CFU/mL (100,000/mL) (Sabljak et al., 2013).

Cottage cheese is likely the oldest and the most common cheese type produced in households in this part of Europe. Its features are a high amount of water and low amount of milk fat with higher level of acidity, as well as specific taste, smell, colour and consistency.

Traditional cottage cheese is usually produced from raw milk left to acidify at room temperature (22°C). Fermenting, in fact curdling, naturally takes 1 to 2 days, but not longer than 3 days (Barukčić et al., 2015). The sour cream is separated and skimmed from the surface of this fermented and curdled milk. Sour milk is poured into a pot and heated at a temperature of 40°C for 2 to 3 hours. The curdled milk is then strained through gauze or a bag with strainer. It can be cut into cubes or leaves and later placed into moulds or other container as described by other authors (Kirin 2009; Landeka et al., 2019).

The main product of the dairy industry in Bosnia and Herzegovina (B&H) is milk, accounting for 64% of overall production, while cheese accounts for 4% (Anonymous, 2017). Another step forward was taken in the milk industry in B&H in 2018, when the European Committee approved export to the EU market for all kinds of dairy products. Production reached nearly 16 thousand tonnes in 2019, up 4% from the year before. An encouraging fact is the positive shift in the structure of cheese production towards cheese types with a longer lifetime, especially hard, semi-hard and slice types (Anonymous, 2020).

Material and Methods

Cheese samples from the most frequent markets in Sarajevo Canton (Markale, Grbavica, Otoka and Ilidža) were taken for microbiological analysis. Ten samples were taken from each market. All samples are the fresh cheese type (cottage soft curd) produced at about 20 registered agricultural households. Microbiological analysis was performed pursuant to the current Regulations and Guidelines for Microbiological Criteria B&H for the following microorganisms: *E. coli*, *Salmonella* spp., *L. monocytogenes*, coagulase positive staphylococci (*S. aureus*), yeasts and moulds. The microbiological criterion was taken as one sampling without subsampling. For analysis, 200 g cottage cheese was taken and transported in transport coolers and delivered for analysis to the laboratory of the Federal Institute for Agriculture – Sarajevo, accredited by the ISO standard 17025.

For identification and counting of microorganisms in cheese, standard ISO microbiological methods of growing agars were used. Sample preparation: 10 g sample and 90 mL sterile peptone solution (homogenisation) → baseline dilutions (10^{-1}). Necessary decimal dilutions are prepared from baseline dilution. For *Salmonella* spp. 25 g sample and 225 mL buffer peptone solution, and for *L. monocytogenes* 25 g sample and 225 mL Fraser-half broth.

For isolation of *S. aureus,* samples of baseline and the second decimal dilution (10^{-1} and 10^{-2}) 0.1 mL was inoculated on Baird-Parker agar and incubated at 37°C for 24–48 hours (EN ISO 6888-1). The coagulase test was used as the confirmation test. For *E.*

Table 1. Criteria of compulsory and recommended microorganisms examined in cheese according to National Regulations of Microbiological Criteria B&H (Anonymous, 2013a) and Guidelines for Microbiological Food Criteria B&H (Anonymous, 2013b)

Cheese type	Microorganisms	Criterion
Soft curd cheese types from raw milk (Cottage cheese)		
Compulsory	*Salmonella* spp.	Not detected in 25 g
	Listeria monocytogenes	Not detected in 25 g
	Coagulase positive staphylococci and other species (*Staphylococcus aureus*)	10^4–10^5 cfu/g
Recommended	*Escherichia coli*	10^2–10^3 cfu/g
	Yeasts and moulds	10^2–10^3 cfu/g
Soft cheese types from pasteurised milk (Cottage cheese)		
Compulsory	*Listeria monocytogenes*	Not detected in 25 g
	Escherichia coli	10^2–10^3 cfu/g
	Coagulase positive staphylococci and other species (*Staphylococcus aureus*)	10^2–10^3 cfu/g
Recommended	*Salmonella* spp.	Not detected in 25 g
	Moulds	10^2–10^3 cfu/g
	Yeasts	10–10^2 cfu/g

cfu/g - (colony forming unit) number of colonies per g

coli isolation of primary and secondary decimal dissolution (10^{-1} and 10^{-2}) 1 mL was inoculated on selective agar is inoculated to selective agar TBX (triptone bileglucuronic) agar and incubated at 44°C for 18–24 hours, and then examined through characteristics matching beta-glucorinidase positive *E. coli* (EN ISO 16649-2). Isolation of *Salmonella spp*. includes pre-enrichment, following the enrichment selective media and then inoculated to selective XLD (xylose-lysine-deoxycholate) agar (37ºC/24 hours) and other media of laboratory choice. Isolation and identification of *Salmonella* includes biochemical and serological tests according to ISO standard (EN ISO 6579-1). For *L. monocytogenes* isolation, half culture medium is used for revitalisation and pre-enrichment listeria. The next level is selective enrichment, followed by inoculation to ALOA and Oxford or Palcalm agar. Characteristics of *Listeria* spp. and *L. monocytogens* colonies are performed on these selective media, and the final identification of *L. monocytogenes* includes ISO standard testing (EN ISO 11290-1). For yeasts and moulds, isolation sample of the baseline secondary decimal (10^{-1} and 10^{-2}) 0.1 mL was inoculated on dichloran-glycerol medium and incubated at 25°C for 7 days, and later examined by characteristics matching yeasts and moulds (EN ISO 21527-2).

Results

An equal number of samples were taken (n=10) from the four most frequent markets in Sarajevo Canton (Markale, Ilidža, Otoka and Grbavica). Out of total number of analysed samples (n=40), 18 samples (45%) were found to be unsanitary, according to National Regulations of Microbiological Criteria for Food in B&H (Anonymous, 2013a) and recommendations from the Guidelines of Microbiological Criteria for Food in B&H (Anonymous, 2013b) adapted to the EU regulations and Directive of Microbiological Food Criteria (2075/201).

Table 2. Results of cheese testing according to microbiological acceptability and pasteurisation

Sample	Microbiologically compliant	Microbiologically non-compliant
Total (40)	22 (55%)	18 (45%)
Pasteurised (24)	19 (79.2%)	5 (20.8%)
Unpasteurised (16)	3 (18.7%)	13 (81.3%)

Table 2 shows the results of the cheese testing according to microbiological acceptability and pasteurisation. The results obtained were statistically analysed using the SPSS software version 19. The results are presented in tables and charts. The accepted level of statistical significance was P<0.05.

Of total of 24 cheese samples produced from pasteurised milk, 19 samples (79.2%) were compliant with the microbiological criteria, i.e., microbiologically suitable. However, five samples (20.8%) were non-compliant according to microbiological criteria, i.e., microbiologically unsuitable. Of the 16 cheese samples produced from unpasteurised milk, three samples (18.7%) were microbiologically compliant while the remaining 13 samples (81.3%) were non-compliant.

Of the total 40 cheese samples, *E. coli* was isolated and counted in four samples (10%) within the permitted limits of microbiological criteria, while the remaining 36 samples (90%) had a value of <10 cfu/g.

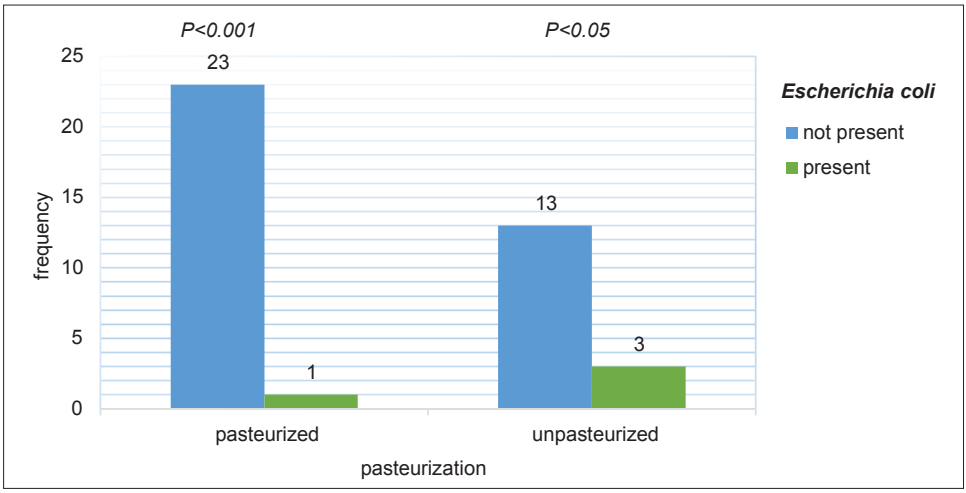

Figure 1. Results of *Escherichia coli* bacteria in cottage cheese samples according to microbiological criteria and milk pasteurisation.

Figure 1 shows the results of testing for the presence of *E. coli* according to milk pasteurisation. Of the 24 samples of cheese produced from pasteurised milk, one sample (4.2%) contained *E. coli* within the permitted boundaries of microbiological criteria, while the remaining 23 samples (95.8%) had values of <10 cfu/g. Of the total 16 cheese samples produced from unpasteurised milk, three cheese samples (18.8%) had isolated *E. coli* within the permitted limits of microbiological criteria, while 13 cheese samples (81.2%) had a value of <10 cfu/g.

Of the total 40 samples of cheese, 14 samples (35%) contained coagulase positive staphylococci (*S. aureus*) at levels higher than the permitted limits of microbiological criteria, while the remaining 26 samples (65%) had value of <10 cfu/g.

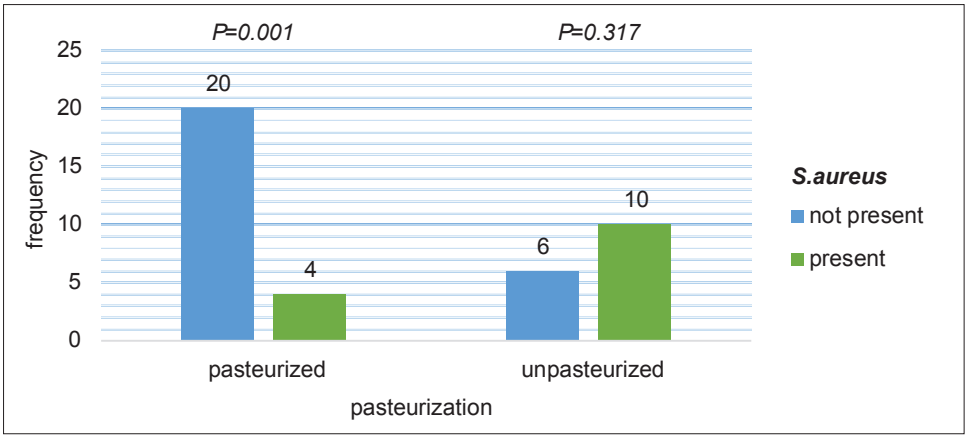

Figure 2. Results of bacteria coagulase positive staphylococci (*Staphylococcus aureus*) in cottage cheese samples according to microbiological criteria and milk pasteurisation.

Figure 2 shows the results of the presence of *S. aureus* in cheese according to milk pasteurisation. Of the 24 cheese samples produced in pasteurised milk, four samples (16.7%) contained *S. aureus* while the remaining 20 cheese samples (83.3%) had a value of <10 cfu/g. Of the total 16 cheese samples produced from unpasteurised milk, ten samples (62.5%) contained *S. aureus*, while the remaining six samples (37.5%) had a value of <10 cfu/g.

Figure 3 shows the analysis for yeasts in cottage cheese. Of the total 40 samples of cheese produced from pasteurised and unpasteurised milk taken from different markets of Sarajevo, 14 samples (35%) contained yeasts within the limits of microbiological criteria, while the remaining 26 samples (65%) had values of <10 cfu/g. The difference in yeast isolation frequency was not statistically significant (P=0.058).

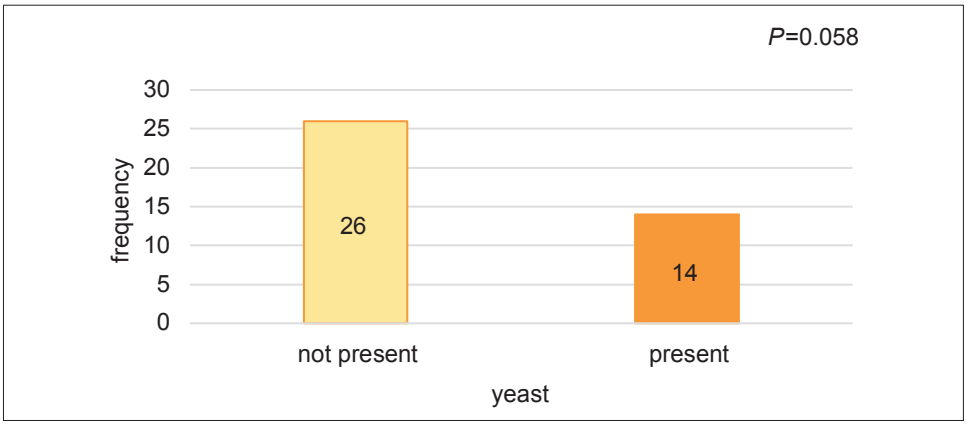

Figure 3. Frequency of yeast presence in cottage cheese samples (within the limits of microbiological criteria).

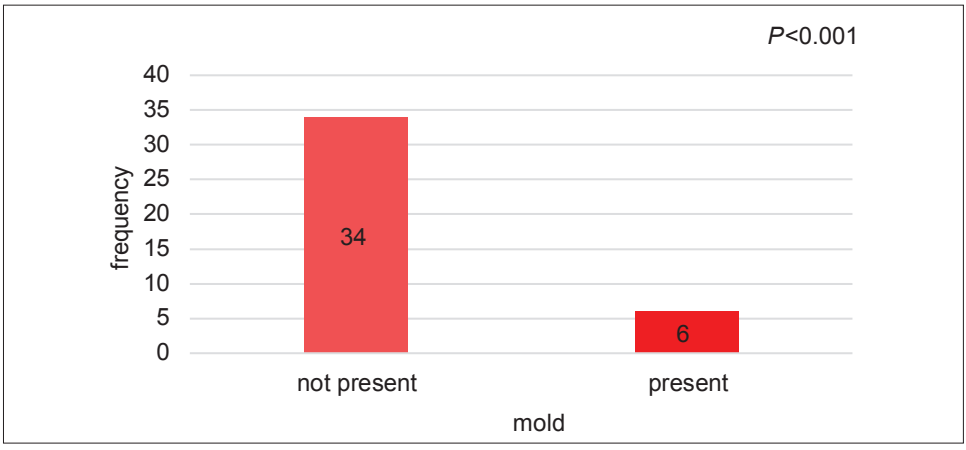

Figure 4. Mould frequency presence in cheese samples (within the permitted limits of microbiological criteria).

Data on mould presence in the examined cheese samples are shown in Figure 4. Of the 40 cheese samples from pasteurised and unpasteurised milk taken from various markets in Sarajevo, six samples (15%) contained moulds and also yeasts within the permitted limits of the microbiological criteria, while the remaining 34 samples or (85%) had values <10 cfu/g. The difference found in the frequency of isolated moulds was statistically significant (P<0.001).

The provisions of the regulations state that in the analysis of soft (fresh) cheese from raw milk, coagulase positive staphylococci and other species (*S. aureus*) is considered negative at a level of <10 cfu/g), acceptable up to 10^4-10^5 cfu/g, or unsatisfactory at >10^5 cfu/g, while for for *E. coli* and yeasts and moulds these levels are negative at <10 cfu/g, acceptable from 10^2-10^3 cfu/g and unsatisfactory at >10^3 cfu/g. Findings for soft (fresh) cheeses from pasteurised milk for coagulase positive staphylococci and other species (*S. aureus*), *E. coli* and moulds are considered negative at <10 cfu/g, acceptable from 10^2-10^3 cfu/g and unsatisfactory at >10^3 cfu/g, for yeasts as negative at <10 cfu/g, acceptable from 10-10^2 cfu/g and unsatisfactory >10^2 cfu/g, while for *Salmonella* spp. and *L. monocytogenes* as n.d. (not detected) as they were not detected in either of these cheese categories.

Discussion and Conclusion

Regardless of milk pasteurisation, of the total number of cheese samples (n=40) used in this study, 22 cheese samples (55%) were indicated as satisfactory concerning microbiological quality, while 18 cheese samples (45%) were indicated as unsatisfactory. Our findings confirmed the presence of *S. aureus* and *E. coli* as the most common bacteria, with detection of moulds and yeasts in the samples, though within the permitted limits. *L. monocytogenes* and *Salmonella* spp. were not detected in the cheese samples. Similar results were reported in other studies. Landeka et al. (2018) compared fresh cheese in the two capitals of B&H and Croatia, where five tested cheese samples (8%) were from pasteurised milk and 55 (92%) of samples were from unpasteurised milk. *E. coli* was detected only in cheese samples from unpasteurised milk, while in this study, *E. coli* was also found in one cheese sample from pasteurised milk together with *S. aureus* isolated in 14 samples (35%).

Research published by Little et al. (2008) in the UK found contamination of fresh cheeses produced from raw or heat processed milk (thermal processing, pasteurisation) by *S. aureus* and *E. coli* bacteria. Unlike our results, that study also confirmed the contamination of fresh cheeses produced from raw milk by *Listeria* spp. (including *L. monocytogenes*) in 3.1% samples i.e., in 2.5% samples of cheeses from pasteurised milk. A study in Ireland (O'Brien et al., 2009) reported the presence of *S. aureus* in 96% of examined samples but within the permitted criteria (<10^5 cfu/g for cheeses from raw milk; <10^3 cfu/g for cheeses from heat processed milk). Cheese containing *S. aureus* in amounts higher than 10^5 cfu/g indicated negative to enterotoxin presence. *E. coli* was present in very small amounts and in less than 10^3 cfu/g, matching the results of our microbiological testing of cheese samples in this study.

Unlike in this study, cheese from a study conducted in northern Africa in Libya (Abujnah et al., 2016) contained bacteria (3% samples). Of the total 87 samples of fresh cheese, 62 samples (71.2%) contained *S. aureus* in levels exceeding the permitted limits. The difference in research results (*E. coli* and *S. aureus* findings) indicates the need for greater sanitary caution during cheese production at higher temperatures, since the research was conducted in a

warmer climate. Positive results for *S. aureus* from the present were rather high compared to a study results from northwest Croatia (Kirin, 2009), where this bacterium was isolated in only 2 of 14 samples (14.29%), while *E. coli* was found in 3 of 14 samples (21.43%). *S. aureus* in cheese can be present as a result of bacterial inflammation of the udder (mastitis) or due to subsequent contamination (Samaržija et al., 2007). These data indicate *S. aureus* and *E. coli* are very common contaminants of fresh cheeses. *Salmonella* spp. and *L. monocytogenes* were not isolated in any samples in the present study. Research in the USA (Abujnah et al., 2016) reported that *Salmonella spp.* was isolated in 7 of 87 samples (8%), while in this study *Salmonella* spp. was not isolated. In research results conducted in the capital of Croatia, Zagreb (Mališa, 2012), *L. monocytogenes* was not isolated from any samples, matching our results, while Markov et al. (2009) reported the presence of *L. monocytogenes* in 10 cheese samples (12%).

Together with *L. monocytogenes*, *Salmonella* spp. is the most common milk pathogen causing serious illnesses in humans (Markov et al., 2009). As for moulds, they were isolated in two samples of cheese from pasteurised milk and four samples of cheese from unpasteurised milk) in this study, which varies from the report of a study by Banjara et al. (2015) who found them in 12 samples (11.36%).

The presence of yeasts and moulds in fresh cheeses is very common and they are the most numerous microorganisms of fresh cheese. Fermentation of these cheeses is performed at room temperature, which is also suitable for the growth of yeasts and moulds. Yeasts and moulds grow at lower pH value than found in fresh cheese (Kirin, 2009). Kirin (2009) reported that yeasts and moulds found in all examined samples were outside the limits of microbiological criteria, while in this study, yeast and moulds were also found in all examined samples, though within the limits of the microbiological criteria. The European Agency for Food Safety established that 4.8% of epidemics transferred by food and occurring in European Union in 2016 were connected to cheeses. In general, this indicates that cheese is a potential source of disease transferred by food, and that *Salmonella* spp., *E. coli* O157:H7, *L. monocytogenes* and *S. aureus* were identified as the main causes of cheese contamination (Ganz et al., 2020).

The research results confirm that the bacteria *S. aureus* and *E. coli* are common contaminants of cottage cheese (fresh cheeses). Their presence may indicate cheese production hygiene, but also milk procedures following milking, production processes, and the implementation of proper hygiene and production practices.

Since fresh cheese is considered a healthy domestic product, it is accepted by consumers and most commonly used in its "natural form", since consumers are now better informed about the food they consume. They should be educated about the production processes, microbiological risks included, and food in general, demanding a minimum of thermal processes, and ensuring that the foods are attractive to the senses and harmless to health. They also have to be aware that fresh cheese, if produced from heat unprocessed milk, may pose a significant public health issue.

The results also indicate that better distribution and selling conditions for this food should be ensured, particularly regarding their sale at open markets in Sarajevo Canton area, with refrigerated showcases set up, as well as in closed markets. Constant, regular and strict control and self-control of the sanitary quality of milk products at all markets is imperative for raising its quality and safety thus protecting consumer health.

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Zastupljenost patogenih mikroorganizama u svježem siru namijenjenog javnoj potrošnji na tržnicama Kantona Sarajevo

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Svježi sir spada u rizičnu skupinu namirnica, a i zauzima najveći segment tržišta mliječnih proizvoda i kao takav se najčešće konzumira kao svježa hrana. Mikrobiološka kvaliteta domaćeg svježeg sira predstavlja potencijalno veliki problem za javno zdravstvo. Cilj je našeg istraživanja bio istražiti i provesti mikrobiološko ispitivanje da bi se utvrdila higijenska ispravnost svježih sireva prikupljenih s najfrekventnijih tržnica u kantonu Sarajevo (Bosna i Hercegovina). Od ukupno *n*=40 analiziranih sireva, 22 uzorka ili (55 %) je bilo higijenski ispravno, dok 18 uzoraka ili (45 %) prema mikrobiološkim kriterijima koji se ispituju u siru prema nacionalnom pravilniku o mikrobiološkim kriterijima Bosne i Hercegovine (BiH) i Smjernicama za mikrobiološke kriterije za hranu BiH nije zadovoljilo mikrobiološku kvalitetu. Ispitivanje je obuhvatilo 24 uzorka pripremljena od pasteriziranog i 16 uzoraka iz nepasteriziranog mlijeka. Međutim, 5 uzoraka sira od pasteriziranog mlijeka ili 20,8 % i 13 uzoraka sira od nepasteriziranog mlijeka ili 81,3 % ocijenjeno je higijenski neispravno. Mikrobiološka analiza napravljena je za obvezne i dio preporučenih mikrooganizama: *Salmonella* spp., *Listeria monocytogenes*, koagulaza pozitivni stafilokoki i druge vrste (*Staphylococcus aureus*), *Escherichia coli* te kvasci i plijesni. Naši nalazi potvrđuju da su najčešće bakterije *S. aureus* i *E. coli*, a pronađeni su i kvasci i plijesni, ali u dopuštenim koncentracijama. *Salmonella* spp. i *L. monocytogenes* u testiranim uzorcima nisu otkriveni.

Ključne riječi: *svježi sir, nepasterizirano mlijeko, pasterizirano mlijeko, Escherichia coli, Staphylococcus aureus, tržnice kantona Sarajevo*