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**Original Scientific Paper****MICROBIOLOGICAL SAFETY AND PHYSICOCHEMICAL QUALITY  
PARAMETERS OF GOAT AND SHEEP CHEESE FROM  
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**Summary**

Cheese is a semi-solid or solid milk product, obtained by curdling milk and separating the curds from the whey. The examination included 46 samples of cheese produced on agriculture farms and small mini-dairies in the territory of Herzegovina, with the emphasis that most of the samples originate from the southern sub-Mediterranean and Mediterranean regions of Herzegovina. The goal of the study was to determine the microbiological safety and physicochemical quality parameters of cheeses that are marketed as fresh goat cheese, semi-solid goat cheese, and sheep and goat bellows cheese. Of the total number of analyzed samples, 49.22% did not meet the requirements of the physicochemical and microbiological parameters of the quality, where 36.22% did not meet the physicochemical quality, and 13% did not meet the microbiological quality due to an increased number of  $\beta$  glucuronidase positive *Escherichia coli*. The presence of  $\beta$  glucuronidase positive *Escherichia coli* in cheese samples indicates insufficient hygiene during the technological process of cheese production itself. Pathogenic microorganisms *Salmonella spp* and *Listeria monocytogenes* were not isolated, and the number of coagulase-positive staphylococci and *Staphylococcus aureus* was within the permitted limits. This study indicates that it is necessary to standardize the physicochemical parameters in the production of cheese, as well as the use of exclusively autochthonous raw materials in the production of individual small producers and in mini-dairies engaged in this production, as is the case with the industrial production of these products in that area.

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**Keywords:** cheese, microbiological safety, physicochemical parameters, Herzegovina

## INTRODUCTION

Milk is the basic ingredient for the production of cheese, and as an essential source of food for the young, it differs significantly depending on the animal species from which it is obtained. In the human diet, fresh milk and milk products are an important source of proteins, fats and energy. Cheese is a food that belongs to the group of finished products and is most often consumed fresh. It is a highly valuable food that is used as a delicacy in most of the world's cuisines. It is a semi-solid or solid milk product, obtained by curdling milk and separating curds from whey. The more whey is separated, the more solid the cheese is. The cheese production process is very complex, for which the selection of milk and cooling to 4°C to complete the bactericidal phase is important. After cooling, the milk needs to be heat-treated and there are two possible ways to do this, thermalization and pasteurization. Cheese can be produced from unpasteurized or pasteurized milk, which depends on the technique and tradition of making it and is very similar in Southeastern Europe, following autochthonous production traditions, with the specifics of production for individual regions and countries of this part of Europe. Bellows cheese is an autochthonous solid milk product from the southern part of Bosnia and Herzegovina, i.e. the sub-Mediterranean part of the Herzegovina region. Its main characteristic is anaerobic ripening inside a bag made of lamb skin (bellow), which is how the cheese got its name. It is produced using traditional cheesemaking techniques from unpasteurized sheep's milk without the addition of a starter culture, and is characterized by a long ripening process, which ultimately gives it specific sensory properties. Lipolysis is dominant and is responsible for taste and smell through biochemical processes during cheese ripening in the animal skin bag, and proteolysis is also involved in the creation of the desired cheese aroma. Compared to cheeses ripened in the natural rind, cheeses ripened in a bag have a uniquely strong and spicy taste. This type of cheese is present in Croatia, Bosnia and Herzegovina, Montenegro and Turkey, with certain differences in the technological production process (Tudor Kalit et al., 2010; Kiš et al., 2018; Rako et al., 2019). The optimal ripening time for cheese from the bag is 45 days, but due to the increased demand for this cheese on the domestic market, the ripening time is very often 30 days (Tudor Kalit et al., 2014; Rako et al., 2019).

Soft cheeses are produced from fresh milk, and fermentation is based on autochthonous microbiota, which consists of lactic acid bacteria (EC, 2003). However, due to hygiene problems, most cheeses today are produced by

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thermalization (65-68°C/5-15 seconds) or pasteurization (72°C/15 seconds) of milk, with the use of selected starter cultures (Meyrand and Vernozy-Rozand, 1999).

Lodi et al. (1994), on the basis of lactic acid bacteria, classified 32 types of fresh cheese into 3 categories: cheeses with a high number of lactic acid bacteria, cheeses with the absence of lactic acid bacteria and a small number of natural microbiota, and cheeses with the absence of lactic acid bacteria, but with a high content of natural microbiota. The authors concluded that in the first category the number of pathogenic bacteria decreases sharply during processing, while in the second and third categories pathogenic bacteria survive for several weeks.

From the aspect of food hygiene, cheese can cause foodborne diseases. Milk and milk products are foods that are often associated with staphylococcal enterotoxin food poisoning (Delbes et al., 2006). However, staphylococcal enterotoxins are not always detected in the final product, although the population of *Staphylococcus aureus* reaches a value over 10<sup>5</sup> cfu/g of cheese, because not all staphylococcal strains are enterotoxic (Delbes et al., 2006; Aoyama et al., 2008). In literature, more and more attention is paid to the contamination of cheese with *Salmonella* and *Listeria monocytogenes*. The prevalence of these pathogenic bacteria in cheese varies from study to study, so that for *Listeria monocytogenes* the prevalence is from 2.1 to 4.8% in cheeses in Italy after packaging (Manfreda et al., 2005). This bacterium gets into the cheese during the production process, so some data suggest that the presence of the bacterium can be inhibited by adding lactic acid bacteria (Ennahar et al., 1998). Also, it is believed that listeria can be found more often in soft cheeses, in contrast to solid cheeses where they are less common. Pathogenic enterobacteria, especially the genus *Salmonella* and *Escherichia coli* (O157:H7) have been detected in fresh cheeses in many studies (Colak et al., 2007).

The most important components in milk solids are milk fat, proteins, lactose, minerals and vitamins. Determination of water is of great importance since it affects the physical, chemical and nutritional properties of the product. The rest represents the proportion of dry matter. One of the basic and very important procedures in the analysis of food and food products is the determination of water. In relation to the proportion of water in the fat-free matter of the cheese, the consistency and structure of the dough, cheeses are produced and placed on the Bosnia and Herzegovina market under the names of extra solid cheese, solid cheese, semi-solid cheese, soft cheese, fresh cheese (Propis, 2011). Milk fat is predominantly composed of triacylglycerols, quantitatively followed by sterols, mostly cholesterol and phospholipids (Chow, 2008).

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The goal of the study was to determine the microbiological safety and physicochemical quality parameters of cheeses from the Herzegovina region, which are marketed as fresh goat cheese, semi-solid goat cheese, and sheep and goat bellows cheese.

## MATERIALS AND METHODS

The cheese samples were produced on agricultural farms and small mini-dairies in the territory of Herzegovina (Bosnia and Herzegovina), with the emphasis that the largest part of the samples originated from the southern sub-Mediterranean and Mediterranean regions of Herzegovina. The characteristics of this area are mostly the extensive way of keeping animals (sheep and goats) and the grass and Mediterranean plants from the karst fields, which the animals eat during milk production. This autochthonous milk serves as a raw material in the production of cheeses from this area. In this study 46 samples of fresh goat's cheese, semi-solid goat's cheese and sheep's and goat's bellows cheese, sampled during three years (2019-2021) were analyzed. The analyses were obtained in the accredited laboratory of the Federal Agro-Mediterranean Institute in Mostar, Bosnia and Herzegovina.

Microbiological examination of cheese samples was carried out according to the current regulations in Bosnia and Herzegovina, the Rulebook on Microbiological Criteria for Food (Regulation, 2013), and the Guidelines for Microbiological Criteria for Food (ASH BiH, 2013), using standard methods, namely:

- BAS EN ISO 6579-1 for the detection of *Salmonella spp.* (ISBIH, 2018a),
- BAS EN ISO 11290-1 for the detection of *Listeria monocytogenes* (ISBIH, 2018b),
- BAS EN ISO 6888-1 for counting coagulase positive staphylococci and *Staphylococcus aureus* (ISBIH, 2005),
- BAS EN ISO 16649-2 for counting  $\beta$  glucuronidase positive *Escherichia coli* (ISBIH, 2008).

Physicochemical examinations of cheese samples were performed using standard methods, namely:

- BAS EN ISO 5534 (ISBIH, 2006) for determining the proportion of water/dry matter,
- AOAC (1996) for determination of milk fat content in cheese.

The proportion of milk fat in the dry matter and the water content in the non-fat matter of the cheese were calculated (Slanovec, 1982).

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## RESULTS AND DISCUSSION

Until these days, cases of cheese contamination with many pathogenic bacteria that can cause human infections are well known and described: *Listeria monocytogenes*, *Staphylococcus aureus*, *Salmonella spp.* and *Escherichia coli* (Kousta et al., 2010). Contamination of cheese most often occurs during the stage of the preparation, transport and storage process, so active monitoring in the production process and in traffic is important for the safety of this food for consumption. In our study, the microbiological examination of cheese samples revealed an unsatisfactory number of  $\beta$  glucuronidase positive *Escherichia coli* in 13% of the samples, exclusively in fresh and semi-solid goat cheese samples ( $>103\text{CFU/g}$  to  $>109\text{CFU/g}$ ), in 6.50% of the samples of cheese, an acceptable number was determined ( $>102\text{CFU/g}$  to  $<103\text{CFU/g}$ ), while 80.50% of the samples were satisfactory ( $<10\text{CFU/g}$ ). The values of coagulase positive staphylococci and *Staphylococcus aureus* were within the prescribed limits. Pathogenic microorganisms *Salmonella spp.* and *Listeria monocytogenes* were not isolated in any of the tested samples. The obtained results are in correlation with research conducted by a number of researchers across Europe. Thus, in the samples of autochthonous bellows cheese from the region of Herzegovina (Bosnia and Herzegovina) produced from raw sheep's and mixed sheep's and cow's milk, *Listeria monocytogenes*, *Salmonella spp.*, *Yersinia enterocolitica* and *Staphylococcus aureus* were not isolated, 40% of the samples contained an increased number of *Escherichia coli*, and Enterobacteriaceae were found in 60 % of the samples (Kiš et al., 2018).

The obtained results regarding *Salmonella spp.* and *Listeria monocytogenes* are in accordance with the results of Golić et al. (2014). The same authors, by examining white sheep's cheese, found an increased number of  $\beta$  glucuronidase positive *Escherichia coli* in 36.40% of the samples, and an increased number of coagulase positive staphylococci in 18.10% of the samples. The finding of colonies of  $\beta$  glucuronidase positive *Escherichia coli* in our case is an indicator of poor hygiene in the production process. Examination of Vlašić cheese originating from Bosnia and Herzegovina, taken from households engaged in the traditional production of this cheese from raw sheep's and cow's milk, with the addition of salt, revealed the presence of coagulase-positive staphylococci in 76% of the samples (Golić et al., 2015). Vlašić cheese supports the growth of staphylococci because it is made from raw milk, which is favored by the production temperature of this cheese (22-30°C), the addition of salt and a ripening period of at least 60 days and up to 180 days. These results differ from ours for the reason that the ripening conditions of Vlašić cheese, due to the mechanism of rennet coagulation, when there is no significant drop in the pH value and the development of lactic acid bacteria, favor

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the growth of staphylococci, which are additionally, indirectly favored by the presence of salt, which prevents the growth of competing bacteria i.e. lactic acid bacteria. When examining Sjenica autochthonous cheese in brine in southwestern Serbia, produced from raw sheep's milk, during the monitored period of cheese production, the number of coagulase-positive staphylococci did not reach the value of 105 CFU/g (Bulajić et al., 2015), which coincides with the results of our study. Also, Zarate et al. (1997) investigated *Staphylococcus aureus* in goat cheese from Tenerife (Spain), produced from raw milk, without the addition of starter, consumed fresh (within 2-3 days) or after ripening for 60 days. A 2-3 log CFU/g increase in *Staphylococcus aureus* was observed in two-day-old cheese, but a rapid decline was noted thereafter.

Water is one of the main ingredients that affects the quality of food during storage, transportation, processing, etc. Consequently, determining the content of water and dry matter is one of the most important methods in food analysis. Table 1 shows the results of the physicochemical parameters of the cheese.

**Table 1** Results of physicochemical parameters of cheese

A type of cheese	Number of samples	Average value and standard deviation				
		DM %	W %	W % <sub>NF</sub>	F %	F % <sub>DM</sub>
Semi-solid goat cheese	22	54.20±5.97	45.80±5.97	57.85±7.22	38.24±10.79	20.70±6.14
Fresh goat cheese	11	44.07±4.47	55.93±4.47	70.45±5.00	46.56±7.15	20.48±3.56
Sheep bellow cheese	7	60.21±3.75	39.79±3.75	56.46±3.58	48.90±6.28	29.53±5.00
Goat bellow cheese	6	63.74±4.35	36.26±4.35	51.80±4.69	47.25±3.02	30.14±3.08

DM % - % dry matter; W % - % water; W %<sub>NF</sub> - % water in non-fat dry matter; F % - % fat; F %<sub>DM</sub> - % fat in dry matter;

The average amount of water with standard deviation in the analyzed samples was 45.80 ± 5.97%, for semi-solid goat cheese, 55.93 ± 4.47% for fresh goat cheese, 39.79 ± 3.75% for sheep's bellow cheese, and 36.26 ± 4.35% for goat bellow cheese. If it is observed by years and category of cheese, this ratio of the average value ranges from 38.88% for semi-solid cheese to 57.18% for fresh goat cheese. The amount of water plays an important role in the formation of the texture of the dough and the internal appearance of mature cheeses. Active water affects the direction of biochemical events, the extent of chemical and physical changes in the dough, the stability of the cheese and its performance. The type of cheese

should be guided by the correct choice of the appropriate raw material, the correct preparation of milk as well as the appropriate technological process. Only in this case can it be expected, with an adequate amount of water in the non-fat content of the cheese, that fermentation processes will take place normally, assuming that all other relevant factors are also normal (Slavonec, 1982). The dry matter that remains after the removal of water is actually the total solids in a food (Nielsen, 2010). The average amount of dry matter with standard deviation in the analyzed samples was  $54.20 \pm 5.97\%$  for semi-solid goat cheese,  $44.07 \pm 4.47\%$  for fresh goat cheese,  $60.21 \pm 3.75\%$  for sheep's bellow cheese, and  $63.74 \pm 4.35\%$  for goat bellow cheese. If it is observed by years and category of cheese, this ratio ranges from 46.26% for fresh goat cheese to 29.53% for sheep's bellow cheese. Milk fat is the mass fraction (m/m) of milk fat in milk, mainly composed of triacylglycerols, quantitatively followed by sterols (mostly cholesterol) and phospholipids. It is believed that milk fat, or the proportion of milk fat, affects the taste of the milk itself. The average amount of milk fat with standard deviation in this study was  $38.24 \pm 10.79\%$ , for semi-solid goat cheese,  $46.56 \pm 7.15\%$  for fresh goat cheese,  $48.90 \pm 6.28\%$  for sheep's bellow cheese, and  $47.25 \pm 3.02\%$  for goat bellow cheese. If it is observed by years and category of cheese, this ratio ranges from 19.20% for fresh goat cheese to 61.64% for goat bellow cheese. The proportion of water in the non-fat content of cheese is one of the parameters according to which cheeses can be divided into different types. The proportion of water affects the ripening of cheese, i.e. with a larger amount of water, rapid chemical changes and faster spoilage occur, due to, among other things, the increased activity of bacteria and enzymes in a moist environment (Nielsen, 2010). The average ratio of water in the non-fat content of cheese with the standard deviation in this study was  $57.85 \pm 7.22\%$  for semi-solid goat cheese,  $70.45 \pm 5.00\%$  for fresh goat cheese,  $56.46 \pm 3.58\%$  for sheep's bellow cheese, and  $51.80 \pm 4.69\%$  for goat bellow cheese. If it is observed by years and category of cheese, this ratio ranges from 52.70% for goat bellow cheese to 73.15% for fresh goat cheese. According to national and international regulations, cheeses are divided into extra-fat, full-fat, fat, semi-fat and low-fat according to the milk fat content, i.e. the milk fat content in the dry matter of the cheese. The average amount of milk fat in dry matter with standard deviation in this study was  $20.70 \pm 6.14\%$  for semi-solid goat cheese,  $20.48 \pm 3.56\%$  for fresh goat cheese,  $29.53 \pm 5.00\%$  for sheep bellow cheese, and  $30.14 \pm 3.08\%$  for goat bellow cheese. If observed by age and category of cheese, this ratio ranges from 16.78% for semi-solid goat cheese to 52.00% for goat bellow cheese. Statistically, expressed through the mean value and standard deviation, all data by cheese category are in accordance with the Ordinance on milk products and starter cultures (Propis,

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2011), while not all specific individual data are aligned with the test parameters for individual cheese categories. Average mean values without standard deviations for the categories of semi-solid goat cheese, fresh goat cheese and sheep's bellow cheese are in accordance with the provisions of the regulations (Propis, 2011). The category of goat bellow cheese at the test level with the average value and standard deviation (Table 1) meets the provisions of the regulations (Propis, 2011). Of the total tested samples, if we take the individual results, the largest number of unsatisfactory samples in all categories of cheese was for the parameter water content in non-fat dry matter 34.07%, which is below or above the prescribed values for the individual category of cheese. Of the tested samples, 2.13% of semi-solid fat goat cheese samples had a milk fat content in dry matter of 16.78%, which is below the prescribed value of a minimum of 25% (Propis, 2011). Almost all small cheese producers correctly declared their products, meeting the provisions of the ordinance (Propis, 2011). Considering the quality of the analyzed samples of all categories of cheese, 36.22% of the samples did not meet the requirements for the parameter water content in non-fat dry matter and fat content in dry matter. The obtained results for physicochemical parameters are similar to the results of other authors. The results of the studies related to cheese among small individual producers, conducted in the area of central and western Bosnia and Herzegovina, for traditional sheep's cheeses (Livanjski and Travnik) are comparable to our results. According to the results of Hrković et al. (2011), the average proportion of dry matter for Livanjski cheese was 62.88%, the water content was 37.11%, and the proportion of milk fat was 31.93%. The result of the average ratio of milk fat for Travnik cheese was 27.08%, and the average ratio of water and dry matter in Travnik cheese was 54.66% and 45.34%, respectively. The results of our tests are consistent with these results, but differ in part in the obtained test values. Mirecki et al. (2015) published the results of study of the physicochemical properties of Njeguški cheese, a traditional sheep's cheese from Montenegro, where they obtained results of milk fat content in sheep's milk of 4.92%, and dry matter without fat of 9.46%. The cheeses belong to full-fat semi-solid cheeses, because they have a fat content of 29.97%, fat in dry matter 51.73%, water in non-fat dry matter 60.07%, and dry matter content 57.93%. This research is in accordance with our study. The results of research conducted by Drozd (2001) in the Tatra region of southern Poland, on the traditional Oscypek cheese, a smoked baked solid cheese produced from raw sheep's milk, with an average water content of 27.50%, a fat content of 22.50% and with an average milk fat content of 31.30%, differ from the results of our study for sheep's milk cheese with a water content of 39.79% and a fat content of 48.90%, which is significantly higher for these test parameters. By

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investigating the physicochemical properties of full-fat, semi-fat and low-fat sheep's cheese in Spain (Sanchez-Marcias et al., 2010), it was concluded that cheeses generally differ due to physical and chemical changes that occur during ripening, and this is primarily influenced by the chemical composition of milk used for cheese production. After fourteen days of ripening, it was determined that full-fat cheese contains 25% milk fat, semi-fat cheese 12.17% milk fat, and lean cheese 4.43% milk fat. The proportion of water after fourteen days in full-fat cheese was 44.12%, semi-fat cheese contained 50.98% water, and lean cheese 55.15%. According to the results obtained in this study, it can be concluded that the values of physical and chemical parameters of cheese are approximately the same.

### CONCLUSION

The results of the microbiological examination indicate the need to improve the hygiene level of the production process, considering the determined number of *Escherichia coli* in the cheese, where special care must be taken when manipulating the milk. Autochthonous Herzegovinian bellow cheese and fresh cheese are usually produced from unheated milk, and it is necessary to apply good hygiene practices, especially preventive procedures, in order to reduce the public health risk for the general population. The risk of *Escherichia coli* is primarily reflected in the sensory characteristics of the cheese, which can endanger the technological production process itself and affect the physicochemical quality parameters. From the physicochemical aspect, the obtained analytical data can represent a contribution to determining the quality requirements and standardization of the production of autochthonous cheeses in the region of Herzegovina. This study indicates that it is necessary to standardize the physicochemical parameters in the production of cheese, as well as the use of exclusively autochthonous raw materials in the production of individual small producers and in mini cheese factories engaged in this production, as is the case with the industrial production of these products in that area. Thus, consumers would be sure of the product they are buying, and the conditions would be met for registration of the quality mark of geographical origin (bellow cheese) at the national level, and eventually at the EU level.

Conflict of interest statement: The authors declare that there is no conflict of interest.

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