The microbiological peculiarities of the telemea cheese (white cheese in brine) obtained in a milk processing unit from Cluj County

Ariana S. (Oprea) Caraba, Marian Mihaiu

Department of Food Inspection and Control, Organizing Institution for Doctoral University Studies, Veterinary Medicine University Cluj Napoca, Romania. Corresponding author: A. S. (Oprea) Caraba, ariana_caraba@yahoo.com

Abstract. Animal products that enter the consumption of the population are in need of continuous improvement, maximization of safety and quality for the consumer and the reduction of production and distribution costs for the manufacturers. Taking into account the recent change in the consumer's mentality and the high demand for products with high nutritional quality, manufacturers are tempted to add traits to the labels of their products which are non-conforming to the contents of the product.

Key Words: cheese, microbiological quality, telemea.

Introduction. To prevent the rise of non-conforming products on the market, the regulations regarding this topic should be stricter and the controls should be more frequent. Cheese, apart from its aforementioned nutritional quality, should also meet a standard of microbiological quality, of hygiene, so that the products can offer benefits to the consumer and not cause food poisoning. Such more common microorganisms which cause food poisoning, although rarely, are the *Escherichia coli* pathogen and *Staphilococcus aureus*. These microorganisms are mostly present in the milk, the raw material, which has been insufficiently heat-treated or in completely untreated milk. The identification of the *E. coli* pathogen germs, which produce the Shigia toxin, is possible in food preparations, including cheese. The research regarding the behavior of the *E. coli* pathogen germs, which produce the Shigia toxin, in ripened cheeses helps us with the assessment of their effects on human health (Miszczycha et al 2013). The risks and benefits of traditional cheeses, especially cheeses manufactured from raw milk, are rarely objectively established. This study begins by highlighting the peculiarities of the microbiota in traditional cheeses, the sensorial and hygiene description, as well as the possible health benefits associated with traditional cheeses. The microbial diversity that forms the basis for the benefits of the cheese manufactured from raw milk depends on the microbiota in the milk, and also on traditional practices, including inoculation practices. Cheeses made from raw milk have a more intense and a richer flavor than processed cheeses. Lactic microorganisms are more diverse in cheeses made from raw milk, compared to those made from pasteurized or microfiltered milk, in which case there are added selected lactic crops, which include only a few microbial species. In the future, the way might be paved for new risk-benefit management methods starting from the farm and ending with the ripened cheese (Monnet et al 2012). The identification of pathogenic germs, such as the *Escherichia coli* pathogen, which produces the Shigia toxin, is currently on the rise when it comes to these cheese varieties. Research teams strive to establish the exact way in which the milk is being contaminated and how it develops in the dairy products, as well as the ways the dairy industry can manage these biological risks, so that the consumer is protected (Farrokh et al 2013). Because of these considerations, in this study we have intended to assess the microbiological risk

represented by *E. coli*, *S. aureus*, staphylococcal enterotoxin, yeasts and molds for the ripened telemea cheese (white cheese in brine).

Material and Method. For the dynamic evaluation of the microbiological parameters found in the ripened telemea cheese, made from cow's milk, there were 126 samples taken from the studied unit, from the following stages of the technological flow: crinta - the placing of the telemea cheese on the cheese mold; the brining; the ripening; the 10-day ripening; the 20-day ripening; the 30-day ripening; and the packaging. For assessing the presence of *E. coli* and *S. aureus* there were 6 samples taken per lot during every studied stage. The samples taken from three distinct lots were packaged in sterile polyethylene bags and transported in isothermal bags at a temperature of 4-6ºC to the laboratory of the Food Inspection and Control, Faculty of Veterinary Medicine, UASVM Cluj Napoca, where the samples were immediately processed.

Processing the samples for the evaluation of the microbiological quality of ripened cheeses. For the isolation and identification of the microorganisms framed within the hygiene category, we used standardized methods, such as: β-glucuronidase positive *E. coli* with SR EN ISO 16649-1/2018, coagulase-positive staphylococci with SR ISO 6888-1:1999/Amd. 2:2018, and yeasts and molds with SR ISO 21527-1/2009. To avoid the contamination of the environment and of the samples, the processing took place on the laminar flow bench and the samples were handled in a manner meant to avoid any risk of contamination. From the collected samples, 10 g were taken and then homogenized in an Easy mix machine for 90 seconds in the presence of 90 mL of buffered peptone water, resulting in the initial suspension (10^{-1}). 1 mL of the base dilution was taken with a sterile pipette, which was then inserted into a test tube with 9 mL of dilution liquid. After closing the test tube, the suspension was homogenized in a Vortex shaker. This resulted in a 10^{-2} dilution. This operation was repeated to obtain the decimal dilutions (10^{-6}). A sterile pipette was used for each dilution. 1 mL of each obtained dilution was placed in the center of 2 Petri dishes using a sterile pipette, before adding approximately 15 mL TBX culture medium (*E. coli*), Baird Parker (coagulase-positive staphylococci), and DRBC (yeasts and molds), which was melted and cooled at 47±1ºC, to obtain a continuous surface. The inoculum and the culture medium were homogenized using rotational movements in a horizontal plane, clockwise movements and counterclockwise movements, and also forward and backward movements. Afterward, the mixture was left to solidify on a cold, flat surface. After complete solidification of the medium, the Petri dishes were kept at 44ºC, for the identification of *E. coli*, and at 37°C for coagulase-positive staphylococci, for 18-24 hours, and at 25°C, and for 24±2 hours for the isolation of the yeasts and molds.

Data analyses. All the obtained results were interpreted using the Origin 8.5 software. The results were interpreted based on the determination of the individual mediums, expressed depending on the standard deviation (n=6). The obtained results were analyzed depending on the following indicators and statistics: arithmetical average, the standard deviation and the probability index. The statistical interpretation of the results depending on the value of the probability index p was carried out as such: $p>0.05$; statistically insignificant results/differences, respectively $p\leq0.05$; statistically significant results/differences, (level of reliability 95%).

Results and Discussion

The evaluation of the microbiological risks in the ripened telemea cheese made from cow's milk. In the analysis of the obtained results, we could observe that the *E. coli* load was in a downward trend during the technological process (Figure 1). Therefore, in the stage of the cheeses' removal from the mould - crinta, *E. coli* showed values ranging from 6 to 120 ufc g^{-1}. After the brining stage, *E. coli* decreased to values ranging from 0 to 25 ufc g^{-1}, with two negative samples. During the 10-day ripening process, *E. coli* showed a slight decrease with values ranging from 0 to 15 ufc mL^{-1}, with three

negative samples. After the 20-day ripening process, only two of the samples contained *E. coli*, with values between 1 and 10 ufc g^{-1}. At the end of the ripening process (30 days), only one sample presented a load of 10 ufc g^{-1}. Similar results were achieved in the packaging stage (Figure 1). It was concluded that from the total number of samples (n=42), 52.4% were negative, which can be explained by the inhibition effect of *E. coli* caused by the salting and ripening process.

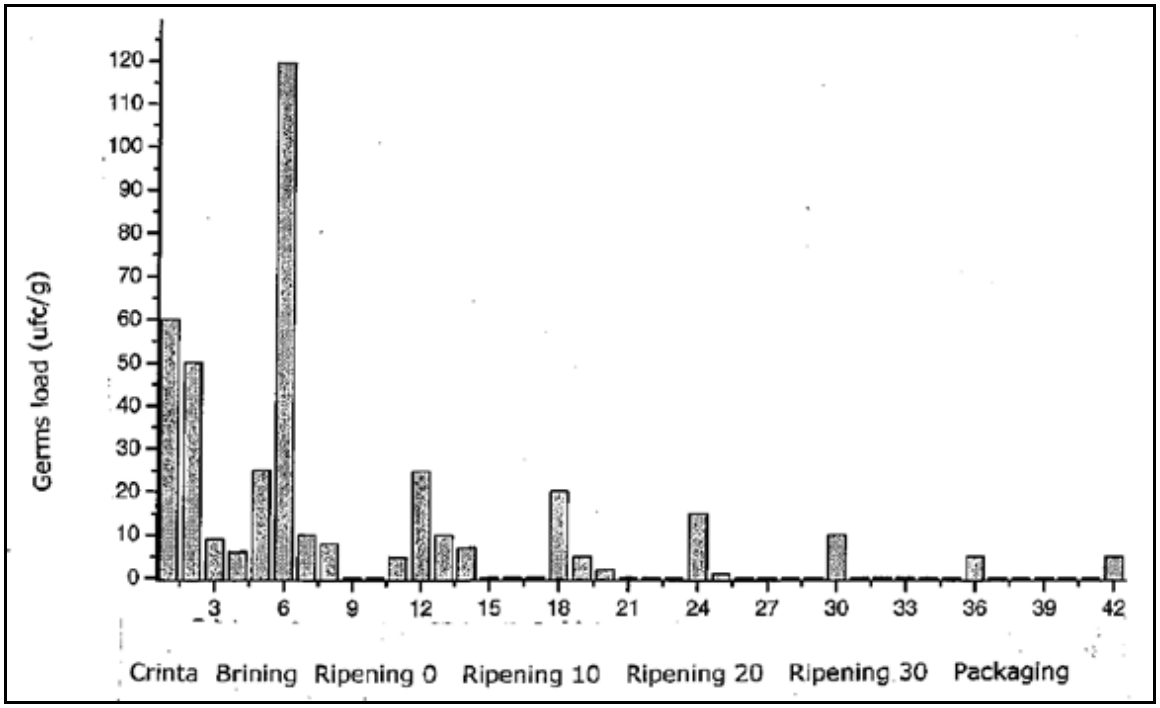

Figure 1. The *E. coli* load during the production process of the telemea cheese (n=42).

The average values of the *E. coli* load during the production process of the ripened telemea cheese are presented in Figure 2. We can observe that the average values of *E. coli* have a downward trend, starting from 45±42.64 ufc g^{-1} when the cheese was removed from the mould - crinta, to 0.8±2.04 ufc g^{-1} at the end of the 30-day ripening process and during the packaging stage. Therefore, at the end of the brining process, the *E. coli* load decreased to 8.0±9.27 ufc g^{-1}. At the end of the first 10 days of ripening, *E. coli* showed values of 3.67±5.89 ufc g^{-1}. Similarly, at the end of the ripening process, the *E. coli* load reached 0.83 ufc g^{-1}, with only one positive sample. Significant differences regarding *E. coli* in the different stages of processing the telemea cheese were outlined between the stages of the cheeses' removal from the mould and the brining ($p<0.01$), between the stage before ripening and the 10-, 20- and 30-day ripening process ($p<0.05$) (Figure 2).

From the analysis of the obtained results, we can assess that all studied samples presented relatively low values, which fall within the limits established by the legal provisions (Reg. 2073 CE/2005), 100 ufc g^{-1}. The initial *E. coli* load showed a significant, steady decrease during the salting and ripening process, which then caused a decrease of the humidity and of the a_w factor (water activity). Suler et al (2010) obtained similar values to the ones of our study, with an average value of the number of colonies ranging from 0.06 to 3.33 ufc g^{-1}, which falls within the limits established by the legal provisions. Therefore, the presence of *E. coli* in these dairy products is due to the hygiene conditions during the technological processing flow (Suler et al 2010; Dan et al 2015). Similar results were presented by Osaili et al (2014), in a study regarding the survival capacity of *E. coli* O157:H7 during the process of preservation in brine. Therefore, it was observed that the *E. coli* O157:H7 load decreased from 7.0 log ufc g^{-1} by 2.6-3.4 log in brine with a concentration of 10, respectively 15% NaCl.

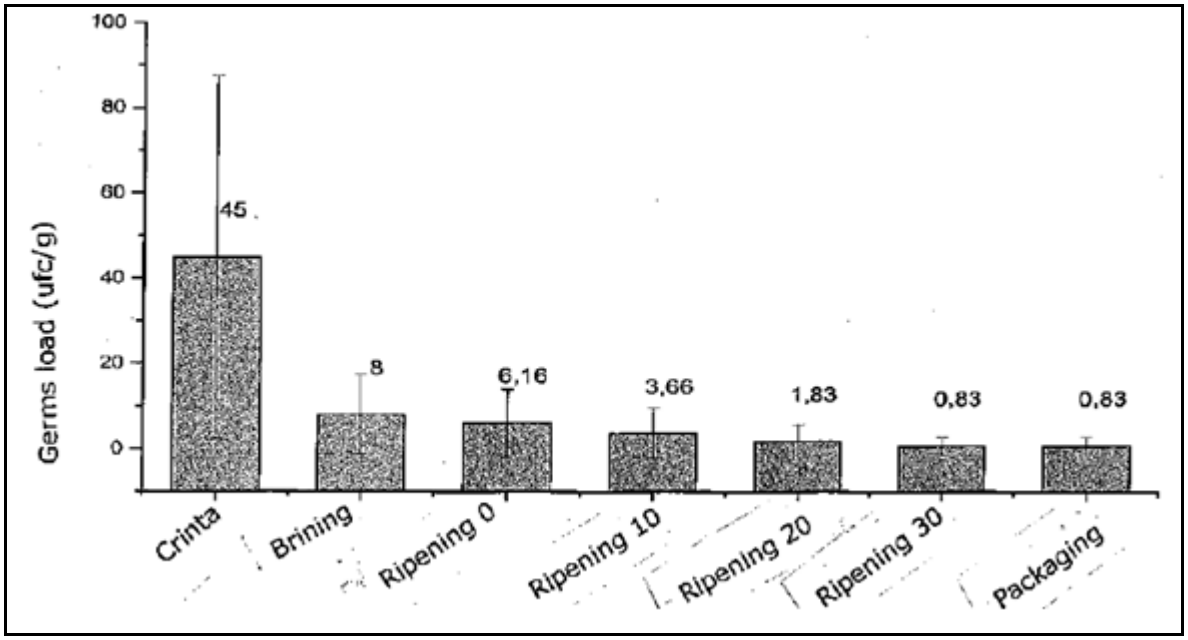

Figure 2. The evolution of the average *E. coli* (±SD) load during the production process of the telemea cheese.

Different results were presented by Mohammadi et al (2009), who observed that *E. coli* O157:H7 increased by 10^6 ufc g^{-1} during the processing of the salted white cheese and decreased during the ripening and salting process to a value of 1.9±0.23 log ufc g^{-1}, in the case of the cheese lots containing the addition of lactic cultures. Similar results were presented by Khayat et al (1988), who observed that from a total of 250 samples tested for coliform bacteria, 46% were negative, and the rest of 54% contained values ranging from 10^2 to 10^7 ufc g^{-1}, over the maximum allowed limits, unlike our results. In the case of values over 100 ufc g^{-1}, the presence of *E. coli* was highlighted in less than 14 hours of ripening. Wusimanjiang et al (2019) have found that *E. coli* O157:H7 and *Listeria monocytogenes* were not affected by the NaCl concentration ranging from 4-10% during the 8-week ripening process, kept at 4ºC. These aspects highlight the potential risk that the *E. coli* and *L. monocytogenes* cultures can have for public health when adapted to low pH conditions and high NaCl conditions. Very high values of the *E. coli* load were specified by Mojsova et al (2013), which highlighted an average value of *E. coli* colonies of 5.3 log ufc g^{-1} (200000 ufc g^{-1}) in the fourth day of ripening, and in the tenth day of ripening, an average value of coagulase-positive staphylococci ranging from 2-4.77 log ufc g^{-1}. After 90 days of ripening, both *E. coli* and staphylococci were not found, which shows that, during this process, the salt concentration and the low value of the pH inhibited the presence of these germs.

The coagulase-positive staphylococci load during the production process of the telemea cheese is presented in Figure 3. We can observe that the coagulase-positive staphylococci load showed a consistent downward trend during the obtaining process, starting at 355±426 ufc g^{-1} at the cheeses' removal from the mould, until the end of the ripening process ($p<0.05$). As opposed to *E. coli*, in the case of staphylococci, all the samples were positive (n=42). Furthermore, 19 of the samples (45.23%) showed values higher than 100 ufc g^{-1}. These results fit into the category of acceptable values, in conformity with the Reg. CE 2073/2005 provisions. One of the samples (2.4%) showed values over 1000 ufc g^{-1}, putting these results into the category of unsatisfactory values. After 10 days of ripening, the coagulase-positive staphylococci load presented a significant decrease, from 247.5±269 ufc g^{-1} to 129±130 ufc g^{-1}. Similarly, 20 days into the ripening process, the coagulase-positive staphylococci load presented a significant decrease, from 129±130 ufc g^{-1} to 88±106 ufc g^{-1} ($p<0.01$). At the end of the ripening process, the coagulase-positive staphylococci load presented a value of 50±77 ufc g^{-1}, significantly lower compared to the values obtained after the telemea cheeses' removal

from the mould. The less accentuated decrease of the coagulase-positive staphylococci in the salting stage is explained by the resistance of these microorganisms in saline mediums. Particularly, *S. aureus* has a high resistance to strongly saline environments, which explains why this pathogen cannot be inactivated by NaCl (Kim et al 2017). The resistance of *S. aureus* was demonstrated through biofilm production (extracellular polymer matrix) in the case of high concentrations of NaCl (Alreshidi et al 2020). Therefore, the forming of biofilm can ensure a protective medium for *S. aureus* to prevent direct contact between the salt and bacteria, thus allowing the bacteria to multiply (Dubois-Brissonnet et al 2016).

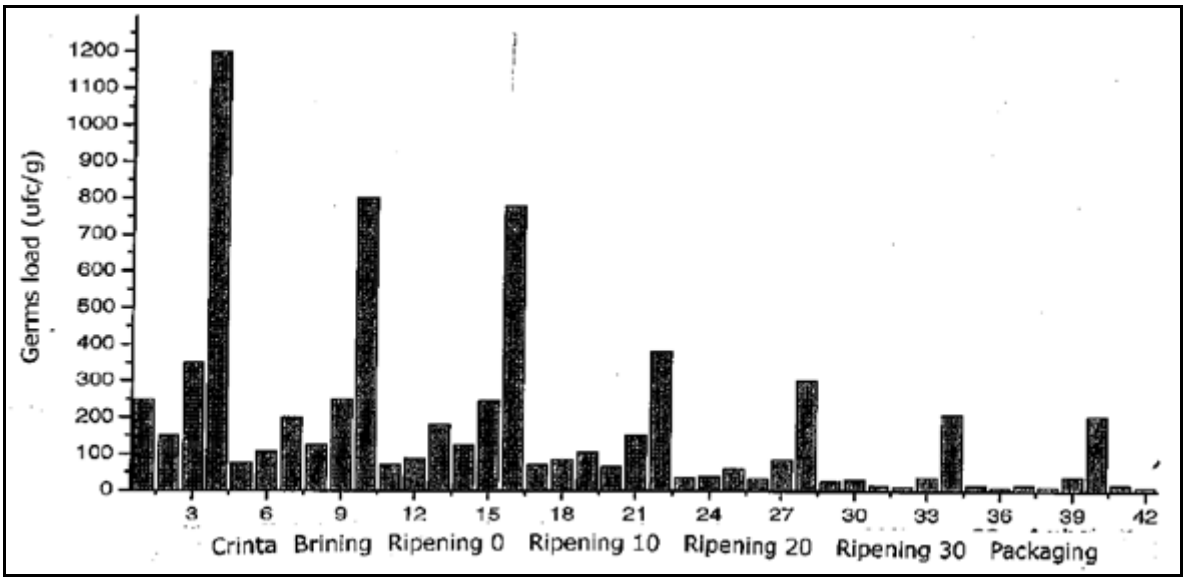

Figure 3. The coagulase-positive staphylococci load during the production process of the telemea cheese.

Considering that the coagulase-positive staphylococci load did not surpass the 105 ufc g^{-1} limit (Figure 4), the testing of the samples regarding the identification of the staphylococcal enterotoxin was not required. Similar values to those obtained in our study were reported by Suler et al (2010), in a study regarding the configuration of the microflora found in telemea cheese, highlighting values of the coagulase-positive staphylococci ranging from 0.48 to 7.08 ufc g^{-1}. Similarly, Khayat et al (1988) found that out of 224 studied samples, only 2% were positive, the rest being in conformity with the applicable legal provisions. Different results from our study were obtained by Duminică (2009), who highlighted a percent of 56.57% *E. coli* and 11.76% coagulase-positive staphylococci in ripened telemea cheese, from the total of studied samples. The same author identified the presence of coagulase-positive staphylococci in a percent of 9.30% in fresh telemea cheese. These values were increased due to the non-compliance with the conditions of preservation of the finished product.

In addition, Al-Nabulsi et al (2020), in a study regarding the factors affecting the viability of *S. aureus* during the processing and ripening of salted white cheese, highlighted that during the 28-day production process, 10% NaCl concentration and 10°C storage temperature caused a 2.26 log ufc increase with starter cultures and 2.96 log ufc g^{-1} without the cultures. When the NaCl concentration increased to 15%, a decrease of the *S. aureus* load was noticed starting on the fourteenth day of the ripening process. In the situation of Pecorino cheese, Lai et al (2020) have found that after 90 days of ripening, *E. coli*, *L. monocytogenes* and *Salmonella spp.* were no longer found in the analyzed samples, showing that the ripening process and salt concentration (similar to the one in the Feta cheese) cause the complete inhibition of pathogenic germs. Only *S. aureus* was isolated at the end of the ripening process, but at very low values, which do not pose a risk for public health.

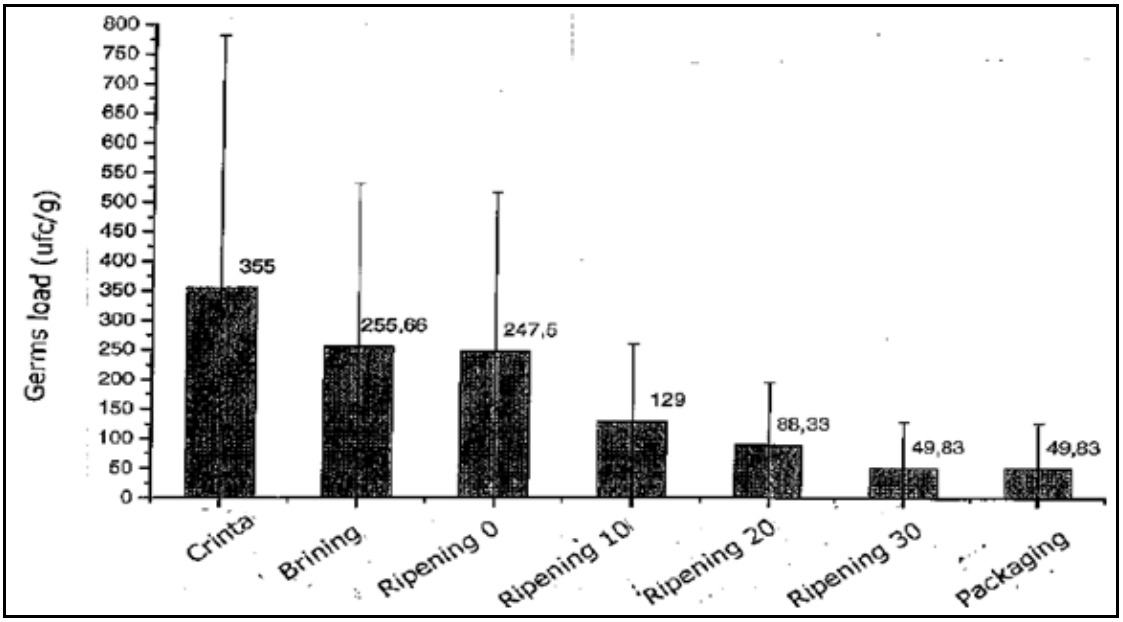

Figure 4. The evolution of the coagulase-positive staphylococci load during the production process of the telemea cheese (n=42).

Figures 5 and 6 present the yeast and mold loads during the production process of the telemea cheese. After analyzing the obtained results, we could observe a downward trend, starting from 1766±2022 ufc g^{-1} in the stage of the cheeses' removal from the mould to 72.5±86 ufc g^{-1} in the packaging stage. Only two of the analyzed samples were negative. Furthermore, five samples (11.9%) surpassed the maximum allowed limit of 1000 ufc g^{-1}. This shows the shortcomings of the sanitation process in the processing areas or a contaminated water source.

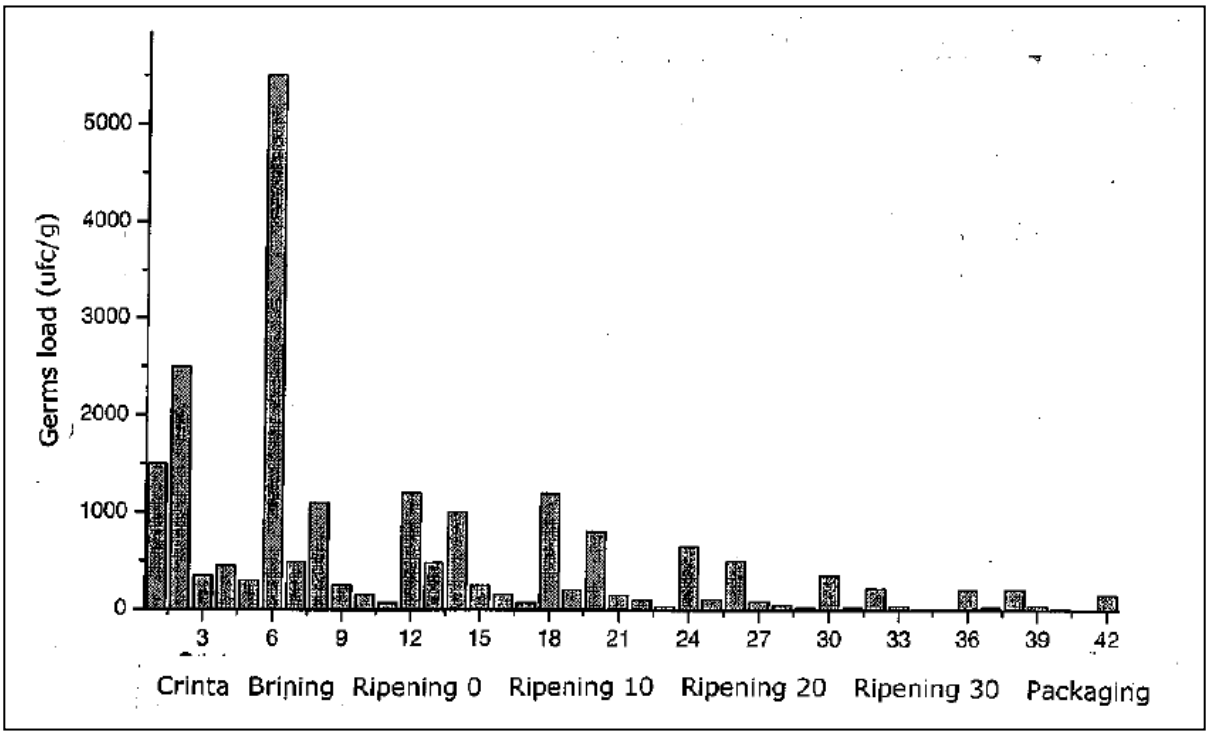

Figure 5. The yeasts and molds load during the production process of the telemea cheese (n=42).

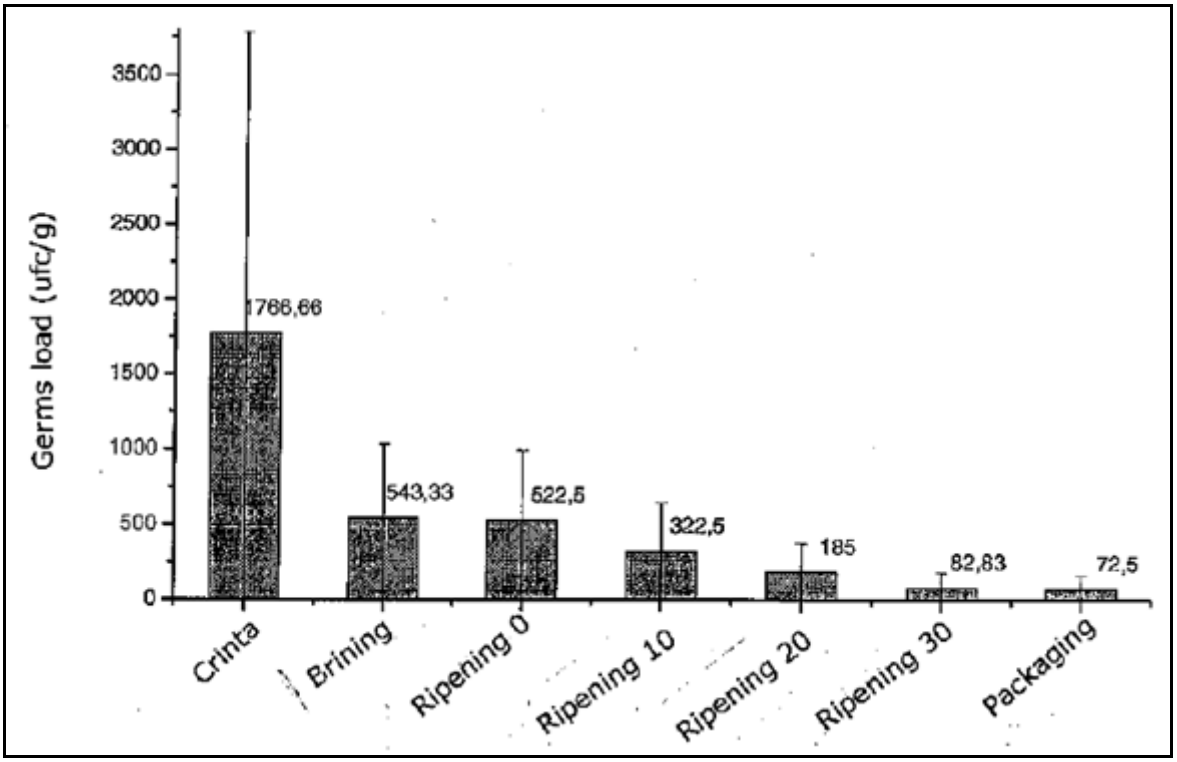

Figure 6. The evolution of the yeasts and molds load during the production process of the telemea cheese (n=42).

Conclusions. As a result of the conducted study, we found that the microbiological risk posed by the presence of *E. coli* and *S. aureus* is low during the ripening process of the telemea cheese. Although the microbial load was relatively reduced, the presence of *E. coli* and coagulase-positive staphylococci indicate deficiencies regarding the enforcement of good hygiene practices in the studied unit. Taking in consideration the presence of *E. coli* and *S. aureus*, we recommend the improvement of the hygiene conditions during the technological process of the fabrication of telemea cheese in the studied unit. Taking into consideration the identification of *E. coli* in some of the lots, which represents an indicator of fecal contamination, the improvement of the hygiene conditions during the fabrication process of telemea cheese is recommended.

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

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Authors:
Ariana Simona (Oprea) Caraba, Department of Food Inspection and Control, Organizing Institution for Doctoral University Studies, University of Agricultural Sciences and Veterinary Medicine, 3-5 Calea Manastur, 400372 Cluj-Napoca, Romania, e-mail: ariana_caraba@yahoo.com
Marian Mihaiu, Department of Food Inspection and Control, Organizing Institution for Doctoral University Studies, University of Agricultural Sciences and Veterinary Medicine, 3-5 Calea Manastur, 400372 Cluj-Napoca, Romania, e-mail: mihaiu.marian@usamvcluj.ro

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