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MICROBIOLOGICAL RISKS IN THE TRADITIONAL PRODUCTION OF WHITE BRINED SHEEP'S CHEESE

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Abstract

In this paper, the microbiological risks in the traditional production of Macedonian white brined sheep's cheese were investigated. The microbiological quality of the raw milk was poor with an average bacterial count (1 680 000 CFU/ml) and somatic cell count (977 000 SCC/ml). Milking and cheese production hygiene was assessed by swabbing and values were expressed in Relative light units. The values obtained were unsatisfactory and ranged from a minimum of 531 RLU in the cheese knife to a maximum of 3 778 RLU in the cheesecloth. The water used in the mountain dairies was mostly contaminated with coliform bacteria and E.coli in 50%, as well as enterococci in 25% of the examined samples. The microbiological analysis of the cheeses after 60 days of fermentation, showed an average of 40 700 CFU/g of aerobic mesophilic bacteria. Of the significant groups of microorganisms from a health point of view, the representatives of Enterobacteriaceae were the most represented in 70%, as well as Escherichia coli in 50% of the examined cheese samples. After two months of cheese ripening, the presence of coagulase positive staphylococci, Salmonella spp and Listeria monocytogenes was not determined.

Keywords: Food safety, cheese, microbiology, traditional production, hygiene.

1. Introduction

White brined cheese is one of the oldest cheeses in the world and belongs to the group of cheeses ripened in brine. Its main distribution area is the Mediterranean Region and the Balkan Peninsula. In North Macedonia, it is a national dairy product with a centuries-old tradition of production using raw milk and basic dairy tools (Mateva et al., 2019). In the past, the production was mostly based on sheep's milk from the indigenous Pramenka breed, including three strains (Ovchepolski, Sharplaninski and Karakachanski). Nowadays, white brined cheese is also produced from cow's and goat's milk.

Given that traditional production of white brined sheep's cheese, involves manual milking of sheep and avoids heat treatment, it provides an ideal environment for the growth of pathogenic microorganisms that can pose risks to human health. Microorganisms usually enter the milk from the environment, the sheep's udder personnel, the air, as well as the utensils and dairy equipment used in production (Mikulášová, 2010)."

The most common types of pathogenic microorganisms encountered in the traditional production of white brined cheese include Salmonella spp., Escherichia coli O157:H7, Staphylococcus aureus, Campylobacter spp., Yersinia enterocolitica, Listeria monocytogenes and Listeria innocua (Tabaran et al., 2016; Bintsis and Papademas, 2002).

The aim of this paper was to assesses the microbiological quality of raw sheep's milk, and determine the hygienic status of milkers' hands, cheesecloth and dairy equipment. Additionally, the study aimed to evaluate the microbiological status of water used in mountain dairies, as well as the microbiological quality of traditionally produced white brined cheeses."

2. Materials and methods

The production of white brined sheep's cheese followed a traditional method at the mountain dairies on Bistra Mountain in North Macedonia, occurring between April and August. This process adhered to the technology outlined in Table 1.

Table 1. Traditional production of white brined sheep's cheese

Step	Technological procedure
1	Milking
2	Filtration through 12-16 layers of cheesecloth
3	Renneting (60 min/30 ^{0}C)
4	Processing of the cheese curd (cutting, mixing, transferring to a cheesecloth)
5	Pressing and draining the whey (4-5 h)
6	Cutting into blocks (11x11 cm) and salting in brine (12 h)
7	Packaging in tin cans with brine
8	Ripening minimum of 2 months (15-17 ^{0}C)
9	Storage (2-4 ^{0}C)

In this study, ten samples of raw sheep's milk were collected after manual milking of the sheep, and the milk's microbiological quality was assessed. Microbiological analyses of the milk included the determination of total bacterial count using the ISO method 21187:2011, as well as somatic cell count detection through the fluoro-opto-electronic method ISO 13366-2:2010.
Hygiene monitoring involved obtaining swabs from the milkers' hands, the milk containers referred to as "vedra," cheesecloth, the cheese kettle, and the cheese knife. UltraSnap ATP tests were utilized for this purpose. Microbiological analysis of the water used in cheese production encompassed the quantification of coliform bacteria and E. coli (ISO method 9308-1), the enumeration of enterococci (ISO 7899-2:2009), the presence of Pseudomonas aeruginosa (16266:2009), the total count of aerobic bacteria at both 22°C and 37°C (ISO 6222:1999), and the assessment of sulphite-reducing anaerobes - clostridia (ISO 6461-2:1986). Cheese samples were examined after a 60-day ripening period. During this period, the following parameters were analyzed: total count of aerobic mesophilic bacteria (ISO 4833), Enterobacteriaceae (21528-2), Escherichia coli (ISO 16649-2), coagulase-positive staphylococci (ISO 6888-1), Salmonella spp. (ISO 6579:2002), and Listeria monocytogenes (ISO 11290-1). The milk and cheese analyses were conducted at the Food Institute of the Faculty of Veterinary Medicine in Skopje, while the water analyses took place in the laboratory of the PHI Center for Public Health in Tetovo.
The acquired research results were processed using the statistical software Statistica 12.0 (Dell, Inc., Tulsa, USA)."

3. Results and discussion

Sheep breeding in North Macedonia boasts a long-standing tradition; however, there has been a noted decline in their population in recent times. Sheep's milk is still procured manually through a practice known as 'straga'. This method involves guiding the sheep from the rear of the sheepfold through narrow passages, where shepherds attend to their milking before allowing them to exit from the front of the fold. This traditional milking approach introduces various types of microorganisms from diverse sources into the milk, thereby posing potential health risks for consumers of dairy products.
As outlined by Mikulášová (2010), primary risk factors in sheep's milk collection include the low milk yield of sheep, the substantial number of animals undergoing milking, subpar hygiene during the milking process, the presence of sheep's wool, and the animals' movement during milking. The most common instances of

milk contamination with bacteria, according to the author, occur during cases of mastitis and fecal contamination, the latter arising when animals defecate during milking.
The European Union, through Regulation 853/2004, has established microbiological criteria for raw sheep's milk. Accordingly, the milk should contain no more than 1,500,000 bacteria per 1 ml. If the raw sheep's milk remains untreated by heat, it must contain no more than 500,000 bacteria per 1 ml to be used in the production of dairy products.
Table 2 displays the results obtained regarding the microbiological and cytological status of the raw milk sourced from the examined herds under mountain breeding conditions. From the acquired findings, it is evident that none of the samples meet the European stipulation that milk should contain fewer than 500,000 CFU/ml. Our results for the total bacterial count align with the observation made by Kalantzopoulos et al. (2002), who assert that in small sheep farms, the average bacterial count seldom falls below 106 CFU/ml.

Table 2. Descriptive statistics for the total bacterial count and somatic cell count in raw sheep's milk (n=10)

	Total bacterial count (CFU/ml)	**Somatic cell count (SCC/ml)**
Average	1 680 000	977 000
Minimum	858 000	710 000
Maximum	3 300 000	1 310 000
SD	849005.2	187937.3
CV%	0.505	0.192

Bogdanovičová et al. (2016) reported a lower average bacterial presence in sheep's milk in the Czech Republic compared to our findings, with a count of 600,000 CFU/ml. However, the authors identified potentially hazardous bacteria species, including Staphylococcus aureus, Listeria monocytogenes, and toxigenic E. coli.
Significant zootechnical measures that could ameliorate the unfavorable microbiological profile of the milk encompass shearing the sheep in areas around the udder, tail, and abdomen. Regular cleaning and disinfection of the milking area, as well as adhering to good hygiene practices among the staff, are of paramount importance. Protecting the sheep from rain is also critical, as milking can lead to the deposition of dirt from the wool into the milk containers.
In addition to the total bacterial count in milk, somatic cell count is a crucial indicator of milk quality. A high number of somatic cells indicates the presence of mastitis in sheep herds. Mastitis can manifest clinically with visible disease signs or subclinically without apparent symptoms. In our study, we found that SCC varies within the range of 710,000 to 1,310,000/ml. While precise thresholds for this parameter are not universally defined, many authors suggest that the number of somatic cells in sheep's milk should not exceed 1,000,000/ml. Tančin et al. (2017) argue that only samples containing over 1,000,000 SCC/ml can reliably signal mastitis in herds.
Mačuhova et al. (2020), who conducted research on various sheep breeds in Slovakia, determined that 56% of samples had SCC below 300,000/ml, while 29% had SCC exceeding 1,000,000/ml. Olechnowicz and Jaśkowski (2005) note that the physiological limit of normal sheep's milk falls between 200,000 and 400,000 somatic cells per ml, escalating to 850,000 SCC/ml in subclinical mastitis, and up to 2,500,000 SCC/ml in clinical mastitis. These authors found that coagulase-negative staphylococci are most commonly present in subclinical mastitis, while Staphylococcus aureus and Escherichia coli predominate in the clinical form.
Based on these scientific observations, it can be inferred that mastitis has occurred in the examined herds. Consequently, it is imperative to enhance hygiene protocols during milking, implement mastitis tests, isolate affected individuals, and administer antibiotic therapy. Furthermore, strict adherence to withdrawal periods for utilized veterinary medications is crucial, as they can adversely impact cheese fermentation, potentially slowing or halting the development of lactic acid microflora. In the traditional production of white brined

sheep's cheese, starter cultures are not employed, making the quality of raw milk and stringent hygiene practices exceptionally vital.

To evaluate both milking and cheese-making hygiene, we employed rapid ATP tests utilizing 10x10 cm square sampling templates. The average results in Relative Light Units (RLU) are presented in Figure 1. Swabs displaying 0-60 RLU signify excellent hygiene of the subject under examination, while readings between 60-180 RLU indicate a dubious status. Results surpassing 180 RLU suggest inadequate hygiene. Separate benchmarks exist for milkers' hands due to the presence of physiological microflora. Hand hygiene is classified as excellent if RLU falls within 0-200, questionable between 200-600, and poor hygiene is indicated by readings exceeding 600 RLU.

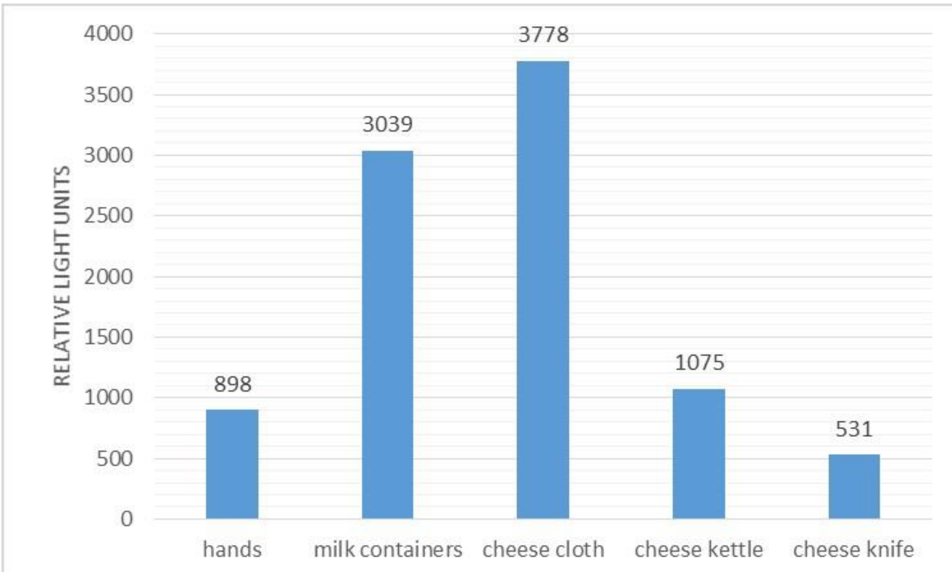

Figure 1. Average RLU values of dairy equipment and hands of milkers (n=50)

Based on the findings presented in Figure 1, it can be inferred that the hygiene standards within cheese production, spanning from the milking staff to the raw milk containers and dairy equipment, have not met satisfactory levels. The elevated Relative Light Unit (RLU) values registered on the milkers' hands (X=898) unequivocally indicate inadequate and insufficient washing practices. This outcome was to be expected, given that the milking staff, who also double as shepherds, typically have limited education and may not prioritize hygiene considerations to the necessary extent. Comparable variability in RLU values was documented during routine hand washing with soap and water for a duration of 30 seconds among workers in food preparation settings, ranging from 116 to 2,281 RLU (Bennion, 2004). In our case, an average RLU value of 3,039 was recorded in the milk containers utilized for manual sheep milking, underscoring inadequate and improper cleaning practices. Comparing our findings to the hygiene status of dairy equipment deployed for colostrum collection in Canadian dairy farms, it is evident that their study indicated a more favorable condition, with a median total data value of 2,958 RLU (Buczinski et al., 2022). Our results align with the conclusions drawn by Roberts and Haslam (2011), who identified normal RLU values in dairy farms ranging between 289 and 896 RLU. Conversely, farms exhibiting hygiene issues displayed high RLU values spanning 2,700 to 30,000 RLU. They propose adopting an RLU threshold of <1000 as a critical marker denoting satisfactory cleanliness of dairy equipment.

Furthermore, the elevated RLU scores detected for the dairy equipment, particularly the cheesecloth (X=3,778), highlight inadequate and insufficient sanitation practices during the equipment cleaning process. Our study revealed that proper alkaline or acidic agents were not employed for equipment cleaning; instead, dish detergents containing anionic surfactants were used, which are insufficient for effectively removing organic debris. The sanitization water was not heated adequately, impairing the removal of fat deposits, and the thermal treatment of the cheesecloth was notably brief. Mechanical cleaning methods proved insufficient, permitting the formation of biofilms from organic matter and the persistence of inaccessible corners, serving as reservoirs for undesirable microorganisms throughout milk processing.

Similar conclusions to those drawn in our study were also reached by Ulusoy et al. (2020). They investigated the traditional production of Halloumi cheese in Northern Cyprus and determined that the RLU values for spoons, cheesecloth, equipment, and cheese tables used in the production surpassed critical thresholds within the range of 20-53%. In the context of mountain dairies engaged in cheese production, untested water sources are employed both for equipment cleaning and brine preparation. This presents a considerable microbiological risk, prompting us to conduct microbiological analyses, the results of which are presented in Table 3.

Table 3. Microbiological analysis of water used in traditional production of white brined sheep's cheese (n=10)

Type of microorganisms	Limits (CFU/ml)	X	Min	Max	% results above limit
Coliform bacteria and *E. coli*	0	2	0	5	50
Enterococci	0	2	0	7	25
Pseudomonas aeruginosa	0	0	0	0	0
Sulfite-reducing clostridia	0	0	0	0	0
Total number of aerobic bacteria at 22 ^{0}C	100	24	7	80	0
Total number of aerobic bacteria at 37 ^{0}C	20	5	0	14	0

The results obtained from the analysis allow us to draw the conclusion that the prevalent source of water contamination was coliform bacteria and E. coli, found in 50% of the samples, with the presence of enterococci detected in 25% of the examined cases. Coliform bacteria, recognized as indicators of fecal contamination in microbiology, signal that water containing such bacteria should be deemed unfit for use due to the associated health risk to humans (Ashbolt et al., 2001). Several authors highlight an elevated microbiological risk in unprotected water sources, particularly during periods of rainfall when fecal matter can infiltrate the water (Mendelsohn and Dawson, 2008). The presence of enterococci and E. coli in our study is likely attributable to wildlife inhabiting the vicinity of the mountain springs, potentially leading to water contamination. This notion is substantiated by research conducted by Probert et al. (2017).

Our research revealed that the water employed in the mountain dairies was devoid of chlorination, and the springs lacked external protection. Consequently, it is imperative to pasteurize the water used in brine preparation, as it holds the potential to harbor microorganisms that could be transmitted into the cheese, thereby fostering premature gas production and cheese spoilage.

In the realm of traditional white brined sheep's cheese production, a multitude of sources house pathogenic bacteria capable of inducing severe health issues, foodborne illnesses, and substantial financial setbacks for producers.

Despite the inherent risks associated with consuming raw sheep's milk cheese, its demand is currently on the rise. This is primarily attributed to the growing prevalence of cow's milk intolerance and the modern trend favoring natural and minimally processed foods (Muehlherr et al., 2003). The microbiological quality of white brined cheeses derived from sheep's milk, produced using traditional methods and subjected to a 60-day maturation period, is detailed in Table 4. The table also presents the critical thresholds as stipulated by the Macedonian Rulebook, which outlines specific requirements for food safety in terms of microbiological criteria (Official Gazette 78/2008).

Table 4. Microbiological analysis of white brined sheep's cheese produced in the traditional way (n=10)

CFU/g	X	Min	Max	Limit	% results above limit
Total number of aerobic mesophilic bacteria	40 700	35 000	46 000		
Enterobacteriaceae	1 130	0	3 100	100 CFU/g	(n = 7/10) 70%
Escherichia coli	2 480	0	7 280	100 CFU/g	(n=5/10) 50%
Coagulase positive staphylococci	0	0	0	100 CFU/g	0
Salmonella spp	0	0	0	0 CFU/25 g	0
Listeria monocytogenes	0	0	0	0 CFU/25 g	0

In our study, the total count of aerobic mesophilic bacteria in the cheese was found to be 40,700 CFU/g (4.07x104), with values ranging from 35,000 to 46,000 CFU/g (3.5x104-4.6x104). These elevated figures can be attributed to various sources of contamination, commencing with raw milk, milk containers, milkers' hands, production rooms, and cheese-making equipment. Notably, high levels of aerobic mesophilic bacteria were also detected in white cheese produced within small, rudimentary dairy facilities, as indicated by Heikal et al. (2014). The authors attribute these findings to the absence of pasteurization and suboptimal hygiene practices concerning the cheese vat, cheesecloth, and curd-cutting knives.

Conversely, the authors did not observe significant counts of aerobic bacteria in the polyethylene bags used for packaging and selling the cheeses. Their study suggests that the packaging material does not represent a noteworthy source of contamination within the examined cheese samples.

Kursun et al. (2008) reported significantly higher levels of aerobic mesophilic bacteria in white cheeses obtained from the markets of Burdur province in Turkey. Their study revealed variations ranging from 1.0x106 to 4.6x109 CFU/g. Similarly, Kara et al. (2020), in their investigation of the microbiological quality of white brined cheeses in Afyonkarahisar province, Turkey, documented an average aerobic mesophilic bacterium count of 2.1x105, with values ranging from a minimum of 4.0x102 to a maximum of 6.0x106. These findings lead the authors to conclude that the total count of aerobic mesophilic bacteria serves as an indicator of hygienic contamination within food production. They also highlight that such counts can escalate under unsanitary conditions of production and storage. Mojsova et al. (2013), in their investigation of Macedonian white brined cheese, observed that during the initial stages of ripening, the cheese exhibited an abundance and wide array of microbial groups. However, as the ripening process progressed, the combination of stringent conditions such as salt concentration, lactic acid, and the lowered pH led to a decline in their numbers. By the culmination of the ripening period, the prevailing microflora within the cheese was predominantly composed of various species of LactobacillusIn the realm of traditional brined cheese production, a paramount factor is the stipulation for the cheese to undergo a minimum ripening period of 90 days. This extended duration serves to eradicate potentially pathogenic bacteria while concurrently reducing the overall bacterial count. Supporting this assertion are the findings of Beev et al. (2019), who demonstrated a noteworthy reduction in the total count of aerobic mesophilic bacteria. Specifically, the count decreased from 45,000 CFU/cm3 at the 24-hour mark to 1,000 CFU/cm3 by the 45th day of production.

The Enterobacteriaceae family encompasses a substantial group of gram-negative bacteria, including members such as Escherichia coli, Klebsiella, Salmonella, Shigella, and Yersinia pestis, which are associated

with various human diseases. In our study, we determined an average count of 1,130 CFU/g of Enterobacteriaceae. Notably, in adherence to regulatory standards, 70% (n=7/10) of the tested samples did not meet the prescribed microbiological criteria for ensuring product safety.
Kara et al. (2020), in their research, reported that the Enterobacteriaceae count ranged from 102 to 106 CFU/g in 34.99% of the examined cheeses, with an average of 1.4x104 (14,000 CFU/g). Additionally, 2.33% of the samples exhibited even higher counts, ranging from 105 to 106 CFU/g. El Zubeir et al. (2006) likewise observed elevated Enterobacteriaceae values in white cheese sourced from three different regions in Sudan, varying between 1.3x104 and 8x104 CFU/g. These disparities can be attributed to diverse production conditions and employed technologies.
In line with findings by Mateva et al. (2019), specific members of the Enterobacteriaceae family tend to be present in substantial quantities during the early stages of ripening in traditionally produced cheeses, with their numbers diminishing as the ripening progresses. Mojsova et al. (2013) underscore the role of Enterobacteriaceae in shaping the distinct organoleptic characteristics of cheeses.
Our results unmistakably underscore the need for heightened attention to hygiene in the traditional cheese production process. This necessitates ongoing education for both shepherds and cheese-makers, as well as enhancements in the hygienic conditions within dairies. Crucially, meticulous and regular cleaning and disinfection of sheep stables and milking areas are essential to mitigate fecal contamination of the milk. Likewise, the sanitation of cheese production and storage facilities must be conducted on a routine basis.
It's evident that the lack of regulated temperature regimes within cheese ripening facilities poses a challenge. Often, white brined cheese ripens at temperatures below 15°C, prolonging the fermentation process and augmenting the potential for undesirable bacterial growth. Therefore, the implementation of controlled temperature conditions is paramount. This not only aids in lowering the pH value of the cheese but also fosters lactic acid fermentation and the production of metabolites that can counteract unwanted bacterial proliferation.
Escherichia coli serves as an important marker for fecal contamination within food and water. According to Bangieva (2020), while this bacterium is generally non-pathogenic, instances of contamination in cheese production typically arise from tainted water sources or unhygienic equipment. Although regarded as non-pathogenic, certain strains like E. coli O157:H7 have the capacity to produce Shiga toxin (STEC), also known as Verotoxin, leading to various human illnesses (Mikulášová, 2010)."
Given the inherent presence of these microorganisms within the gastrointestinal tract of sheep, it becomes paramount to prevent the contamination of milk with fecal matter during manual milking procedures. Notably, a series of reports from the European Union's Rapid Alert System for Food and Feed (RASFF) documenting the detection of Shiga toxin-producing E. coli (STEC) in raw sheep's milk cheese has sparked significant concern (Condoleo et al., 2022). Consequently, tests for the presence of E. coli in indigenous dairy products assume utmost importance.
Our study yielded an average E. coli count of 2,480 CFU/g in the cheese, with a prevalence rate of 50% (n=5/10) for the presence of this bacterium. In accordance with North Macedonian food safety regulations, the presence of E. coli in cheeses sold within markets is strictly prohibited. These outcomes serve as indicators of subpar hygiene practices and inadequate measures during milking and cheese production processes.
Similar results mirroring ours were observed by Ulusoy et al. (2020), who examined the traditional Halloumi cheese. Additionally, Bangieva (2020) reported the presence of E. coli in cow's cheese derived from raw milk, observing a decline in bacterial counts after 30-45 days of cheese ripening. Bangieva attributes this reduction to factors such as diminished water activity, elevated salt content in the brine and cheese, and the lowered pH values of the cheese. In a study conducted by Kara et al. (2020), the counts of Escherichia coli in white brined cheeses ranged from 102 to 105 CFU/g, with this bacterium detected in 10% of the samples.

Therefore, in the production of cheeses, it is important to control the strength of the brine, salt content in the cheeses, the dynamics of active and titratable acidity, as well as the environmental conditions in the cheese ripening rooms.

In our study, we conducted a microbiological analysis of the cheese after a 60-day period, and the presence of E. coli serves as a clear indication that these cheeses are unsuitable for consumption during that timeframe. The consumption of unripened cheeses can potentially lead to a variety of health issues in humans. Hence, it becomes imperative to conduct further microbiological analyses on cheeses produced from raw sheep's milk, while also extending the ripening period.

Coagulase-positive staphylococci may be present in both milk and cheese, capable of causing health disturbances in humans. Among this group of bacteria, Staphylococcus aureus emerges as the most significant representative. Enterotoxigenic strains of this bacterium produce toxins that can induce staphylococcal poisoning, posing a substantial risk to human health when pathogen levels exceed 5 log10 CFU/g (Bangieva, 2020).

The presence of Staphylococcus aureus in milk can stem from instances of mastitis, inflammation of the mammary gland in animals. Additionally, research has established that personnel can also serve as a source of contamination during cheese production. This transmission can occur through actions such as coughing, sneezing, or contact with purulent infections on hands. Moreover, inadequate hygiene conditions and equipment sanitation can further facilitate the propagation of this bacterium (Heikal et al., 2014).

In our study, the presence of coagulase-positive staphylococci in the final products was not detected, likely due to the unfavorable conditions for the proliferation of these bacteria during the cheese fermentation process. Our findings align with the assertions of Mikulášová (2010), who underscores that milk pasteurization effectively eliminates S. aureus, while cheese fermentation acts as an impediment to their growth. Similar observations of low coagulase-positive staphylococci levels were made in mature brined Telemea cheese in Romania by Tabaran et al. (2016). Their study reported values ranging from 1±1.73 to 45.66±6.02 CFU/g, which adhered to the legal requirement stipulating a maximum count of 100 CFU/g.

However, it remains essential to maintain regular microbiological oversight of cheese produced using traditional methods, as several studies have documented the presence of S. aureus in the brine or cheese. For instance, Ulusoy et al. (2020) reported counts ranging from 1.1x103 to 2.3x104 CFU/g of S. aureus in mature Halloumi cheese. Heikal et al. (2014) detected this bacterium in 6.7% of examined samples of white cheese in Egypt, with an average count of 4.37 Log CFU/g. Similarly, Kara et al. (2020) determined an average count of 4.1x102 coagulase-positive staphylococci in cheeses from the local market.

Salmonella spp. frequently contribute to cases of foodborne illness, with instances of such cases emerging after the consumption of raw milk cheeses, although a higher incidence has been observed following the consumption of eggs, poultry, and poultry products (Mikulášová, 2010). Salmonella, being Gram-negative, motile, facultatively anaerobic bacteria, tend to thrive within a temperature range of 8-45°C and a pH of 4-8. They are categorized as zoonotic or potentially zoonotic pathogens. Our cheese examinations, however, did not reveal the presence of these bacteria in any of the samples, despite numerous studies detecting them. Bintsis and Papademas (2002) indicate that the primary limiting factors restraining the proliferation of this bacterium in white brined cheeses are the elevated salt concentrations and the low pH value below 5 units.

Heikal et al. (2014) identified the presence of Salmonella in 6.7% of Egyptian white cheese samples, attributing the contamination primarily to raw milk and unhygienic handling practices. Furthermore, Beev et al. (2019) highlighted the substantial reduction of Salmonella spp. through milk pasteurization, with their count plummeting from 800 CFU/ml in raw milk to 2 CFU/ml in pasteurized milk. They also noted that while Salmonella was present at a level of 20 CFU/cm3 in fresh cheese after 24 hours, they were undetectable in mature brined cheese after 45 days of ripening.

Listeria monocytogenes, a significant pathogenic bacterium, is responsible for a range of illnesses in mammals, birds, and fish. In the human population, the health issues it causes can vary from mild symptoms like diarrhea and headaches to invasive listeriosis with a high mortality rate. Given its presence in soil, water, plants, and farm environments, the risk posed by this pathogen is substantial, making its control in food production crucial (Mikulášová, 2010).

In our study, the presence of L. monocytogenes was not detected in the cheese after a 60-day ripening period. Similar findings were reported by Mojsova et al. (2013) in traditional Macedonian white brined cheese. They attribute this outcome to the presence of enterococci producing bacteriocins in the cheese, which had an inhibitory effect on L. monocytogenes. In research by Beev et al. (2019) and Bangieva (2020), L. monocytogenes was likewise absent from raw milk and cheese at all stages of ripening. As highlighted by Bintsis and Papademas (2002), controlling this bacterium is vital due to its resilience to acidity, salt, and low temperatures. The authors point out that L. monocytogenes survived for 90 days in Feta cheese stored at 4°C with a pH of 4.3. Since this bacterium can tolerate salinity and thrive in brine, pasteurizing the brine is recommended to ensure a safe product.

In a study by Bogdanovičová et al. (2016), L. monocytogenes was detected in 4.4% of sheep milk samples. They underscore that raw milk can pose health risks to consumers and recommend pasteurization as the optimal choice in dairy production. Traditional sheep cheeses made from raw milk can potentially harbor dangerous pathogenic microorganisms, emphasizing the need to enhance Good Manufacturing Practices (GMP) and Good Hygienic Practices (GHP) throughout all production stages.

4. Conclusions

This study underscores that the production of traditional white brined cheeses in North Macedonia is faced with notable risk factors, including the microbiological quality of raw milk, the personal hygiene of milkers, water microbiological quality, and equipment hygiene. Consequently, a more frequent education program for personnel engaged in production, steadfast adherence to sanitation and hygiene practices, and routine inspections of production facilities by state regulatory bodies are imperative.

References

[1]. Ashbolt, N. J., Grabow, W., Snozzi, M. 2001. Indicators of microbial water quality. In: Fewtrell L, Bartham J, editors. WHO Water Series. Water quality-Guidelines, Standards and Health: Assessment of risk and risk management for water-related infectious disease. London: IWA Publishing; p. 289–315.
[2]. Bangieva, D. R., 2020. Microbiological and physicochemical changes during ripening in Bulgarian white brined cheese made from raw cow milk. Bulgarian Journal of Veterinary Medicine, 23(4), 494-503.
[3]. Beev, G., Kolev, T., Naydenova, N., Dinev, T., Tzanova, M., Mihaylova, G. 2019. Physicochemical, sanitary and safety indicators changes during the ripening of Bulgarian white brined cheese from local farms. Bulgarian Journal of Agricultural Science, 25(3), 109–115.
[4]. Bennion, N. 2004. Evaluation of handwashing efficiency. International Food Hygiene, 17(3), 23-24.
[5]. Bogdanovičová, K., Vyletělová-Klimešová, M., Babák, V., Kalhotka, L., Koláčková, I., Karpíšková, R. 2016. Microbiological quality of raw milk in the Czech Republic. Czech Journal of Food Science 34,189–196.
[6]. Bintsis, T., Papademas, P. 2002. Microbiological quality of white-brined cheeses: a review. Society of Dairy Technology, 55 (3), 113-120.
[7]. Buczinski S., Morin, M. P., Roy, J. P., Rousseau, M., Villettaz-Robichaud, M., Dubuc, J. 2022. Use of ATP luminometry to assess the cleanliness of equipment used to collect and feed colostrum on dairy farms. Journal of Dairy Science, 105(2), 1638–1648.
[8]. Condoleo, R., Palumbo, R., Mezher, Z., Bucchini, L., Taylor, R.A. 2022. Microbial risk assessment of Escherichia coli shiga-toxin producers (STEC) in raw sheep's milk cheeses in Italy. Food Control, 137, 108951.
[9]. El Zubeir, I. E., Sanna, B. E. Z., Sanaa, O. Y. 2006. Occurance of Enterobacteriaceae in Sudanese White Cheese in Restaurants of Khartoum State (Sudan). Microbiology, 1(1), 76-82.
[10]. Heikal, G. I., Khater, D. F., Al-Wakeel, S. A. 2014. Bacteriological hazard of white cheese processed in dairy shops in Tanta city. Benha Veterinary Medical Journal, 26(1): 185-194.
[11]. Kalantzopoulos, G., Dubeuf, J.P., Vallerand, F., Pirisi, A., Casalta, E., Lauret, A., Trujillo, T. 2002. Characteristics

of the sheep and goat milks: Quality and Hygienic stakes for the sheep and goat dairy sectors. IDF SC on Microbiological hygiene, Agenda item 4.8.

[12]. Kara, R., Acaroz, U., Gurler, Z., Zengin, Z. S., Soylu, A. 2020. Determination of microbiological quality of white cheeses marketed in Afyonkarahisar. International Journal of Sustainable Agricultural Research, 7(2), pp. 93-97.

[13]. Kursun, Ö., Güner, A., Kırdar, S.S., Akcan, K., A.C. 2008. Determination of microbiological quality of white cheese consumed in Burdur. 10th. Food Congress, Erzurum, Turkey (in Turkish).

[14]. Mačuhová, L., Tančin, V., Mačuhová, J., Uhrinčať, M., Oravcová, M., Vršková, M., Tvarožková, K. 2020. Effect of somatic cell count on milkability and milk composition of ewes. Potravinarstvo Slovak Journal of Food Sciences, 14, p. 1035-1041.

[15]. Mateva, N., Levkov, V., Srbinovska, S., Santa, D., Mojsova, S., Sulejmani, E. 2019. Characteristics of Traditional Cheeses Produced in the Republic of North Macedonia. Current Developments in Food and Nutrition Research, 1, 1-52.

[16]. Mendelsohn, J., Dawson, T. 2008. Climate and cholera in KwaZulu-Natal, South Africa: the role of environmental factors and implications for epidemic preparedness. Int. J. Hyg. Environ. Health, 211(1-2):156-162.

[17]. Mikulášová, M. 2010. Microbial risk of products made from raw sheep milk. Zenodo. https://doi.org/10.5281/zenodo.819743.

[18]. Mojsova, S., Jankuloski, D., Sekulovski, P., Angelovski L., Ratkova, M., Prodanov, M. 2013. Microbiological properties and chemical composition of Macedonian traditional white brined cheese. Macedonian Veterinary Review, 36, 13-18.

[19]. Muehlherr, J. E., Zweifel, C., Corti, S., Blanco, J.E., Stephan, R. 2003. Microbiological Quality of Raw Goat’s and Ewe’s Bulk-Tank Milk in Switzerland. J. Dairy Sci., 86, 3849–3856.

[20]. Official Gazette of the Republic of Macedonia, 78. 2008. Rulebook on special requirements for food safety in relation to microbiological criteria.

[21]. Olechnowicz, J., Jaśkowski, J. M. 2005. Somatic cells in sheep milk. Medycyna Weterynaryjna, 61(2), 136-141.

[22]. Probert, W., Miller, G. M., Ledin, K. E., 2017. Contaminated Stream Water as Source for Escherichia coli O157 Illness in Children. Emerg Infect Dis. 23(7): 1216–1218.

[23]. Regulation (EC) No 853/2004 of the European Parliament and of the Council of 29 April 2004 laying down specific hygiene rules for food of animal origin. Official Journal of the European Union, L 139, 30.4.2004, p. 55–205.

[24]. Roberts, J., Haslam, M. 2011. Use of the 3MTM Clean-Trace surface ATP test to assess parlour hygiene and investigate bactosan breakdowns. In: Pocknee, B. (ed). British Mastitis Conference. Sixways, Worcester, pp. 57-58.

[25]. Tabaran, A., Dan, S.D., Reget, O., Cordea, D.V., Magdas, A., Mihaiu, M. 2016. Microbiological Quality Evaluation of Various Types of Traditional Romanian Cheese through Advanced Methods. Bulletin UASVM Veterinary Medicine, 73(2), 418-424.

[26]. Tančin, V., Baranovič, Š., Uhrinčať, M., Mačuhová, L., Vršková, M., Oravcová, M. 2017. Somatic cell count in raw ewes' milk in dairy practice: frequency of distribution and possible effect on milk yield and composition. Mljekarstvo, 67(4), p. 253-260.

[27]. Ulusoy, B.H., Hecer C., Berkan S. 2020. Investigation of Microbiological Hazards in Traditional Halloumi/Hellim Manufacturing Process. Atatürk University J. Vet. Sci., 15(3): 196-206.