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MICROBIAL DIVERSITY IN TRADITIONAL CHEESES FROM NORTH MACEDONIA: INSIGHTS FROM EXISTING STUDIES

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ABSTRACT

Study examines the diversity and role of microorganisms in cheeses based on existing studies, with a particular focus on traditional Macedonian cheeses. Kashkaval, white brined cheese and beaten cheese are the most common cheeses produced by small farms and farmhouses in North Macedonia. These cheeses are distinguished by their unique production methods, which result in different physical, chemical, and microbiological profiles. A variety of microorganisms, such as bacteria, yeasts, and molds, play an important role in the ripening process, affecting the process of proteolysis, texture, and volatile compounds. In particular, *Lactobacillus* species such as *Lb. plantarum* and *Lb. acidophilus* are prevalent at different stages of cheese ripening. The cheeses are traditionally made from raw milk without commercial starter cultures, relying on indigenous lactic acid bacteria. Despite challenges related to milk quality and safety, local food initiatives remain crucial for the sustainability of the dairy subsector in North Macedonia. The study recommends strengthening cooperation among stakeholders, promoting traditional products, establishing support mechanisms, and implementing educational programs to improve milk quality and safety. Further research is needed to ensure microbial diversity preservation in traditional cheeses that should investigate artisanal cheeses in North Macedonia using genomic and metabolomic methods to gain insights into their microbiological and chemical properties. Collaborative efforts are essential to support local dairy producers and implement strategies to improve the dairy sector in North Macedonia. This will contribute to the protection of traditional cheeses and their microbial biodiversity.

Key words: Microbial diversity, Lactic acid bacteria, Traditional cheeses, Dairy sector.

INTRODUCTION

Throughout history, cheese has been a culinary staple and a product of elaborate scientific and artisanal processes. Cheese undergoes a series of microbiological and physicochemical transformations. Fermentation is vital to the production of beverages like wine and beer, as well as cheese, extending its shelf life and enhancing its sensory qualities. As a result of the adaptability of the microorganisms used in the process, there are many types of cheese available. A remarkable amount of live, metabolic bacteria can be found in cheese (Eck & Gillis, 2000). Microorganisms, primarily bacteria and fungi, play an important role in cheese making. The presence of these microorganisms contributes to the flavor, texture, and other characteristics of cheese (Lessard et al., 2014). Milk, bacteria, salt, and enzymes are just a few basic materials that can be used to produce dozens of cheese variations. Fermentation is a process for storing food while also significantly altering it in ways that depend on the metabolism of bacteria. In large part, microbial fermentation is an enzyme-dependent process

in which the microbes' protein catalysts break down complex substances in this case, the proteins and sugars in milk-into simpler products that can typically be stored for longer periods of time than the original substance**.** Fresh cheeses can be eaten right away after production, but aged cheeses must ripen before they can develop the specific flavor, aroma, and texture that are unique to that cheese. Both the milk's proteins and sugars change when cheese is made. In specifically, fermentation, which depends on a species of bacteria known as lactic acid bacteria (LAB), converts the milk sugar lactose into lactic acid. Traditional cheese producers have relied on the environment and bacteria found in milk to produce their cheeses for ages. Microorganisms from raw milk, cheese-making equipment, and surroundings are found in raw milk cheeses. Without additional starters, these naturally occurring LAB microbes initiate fermentation. Flavour and texture are impacted by non-starter LABs that form during the ripening of cheese. Cheeses made from raw milk have valuable bacteria that can posse valuable technological or human-health related functional properties (Gantzias et al., 2020). Based on previous research on traditional Macedonian cheeses, the goal of this study is to investigate the diversity and significance of microorganisms in cheese. It also looks at the dairy sector's current position and difficulties. Finally, the authors share their observations and conclusions.

MICROBIOLOGICAL DIVERSITY AND CHARACTERISTICS OF MACEDONIAN CHEESES

Kashkaval, white brined cheese, and beaten cheese ("bieno sirenje") are among North Macedonia's dairy heritage. Farms and farmhouses produce most of these cheeses. Physical, chemical, and microbiological profiles of these cheeses differ due to their specific production methods. Due to their microbial content, these cheeses have unique flavors, textures, and tastes. Their production is notably prevalent in the mountainous and rural areas. The absence of commercial starter cultures means that production is dependent on the indigenous lactic acid bacteria present in raw milk. As cheese production technology advances, there are opportunities to refine and enhance the properties of these traditional cheeses, potentially impacting their microbial diversity. Previous studies investigated the physicochemical and microbiological properties of traditional white brined and beaten cheeses (Levkov et al., 2014; Levkov and Kakurinov, 2012; Mojsova et al., 2013; Sulejmani et al., 2014).

Beaten cheese (Bieno sirenje)

Beaten cheese on Macedonian “Bieno sirenje” has its origins in the central area of Mariovo region, with its production dating back to the Ottoman Empire. There are still places where homemade production is still alive, despite industrial production. Today, it's mostly made with cow's milk (Talevski, 2012). Industrially manufactured from cow's milk with starter cultures, this cheese is also traditionally made from raw cow's and sheep's milk. Most of it is produced by family farms or farmhouses in North Macedonia. They are typically sold at green markets or directly from farms. A semi-hard cheese, with yellowish color with varied hole patterns when cut. The cheese undergoes a unique "beating" process where the curd is broken into small pieces using a specialized device, giving the cheese its name. The cheese is placed on a cloth-covered wooden table to ripen at room temperature for 2-7 days or it can be exposed to the sun ("sunning") for 2-3 days, during which time the cheese takes on a yellow colour caused by the milk fat that has come to the surface. the cheese is left to ripen for 30-45 days. (Mateva et al., 2019; Sulejmani et al., 2014)

The study conducted by Levkov et al., (2014) focused on the production of traditional beaten cheese in two farms situated in the Mariovo region of southern Macedonia during the summer using raw ewe's milk. The milk showed high microbial counts, including aerobic mesophilic bacteria, presumptive lactococci, presumptive lactobacilli, coliform bacteria, and yeasts. The predominant microbial group during cheese production and ripening was lactic acid

bacteria (LAB), with their counts peaking during dry ripening and then declining during salting and brining. A total of 240 LAB isolates were identified throughout the manufacturing and ripening stages. The prevailing LAB species were *Lactococcus lactis* ssp. *lactis* (35%), *Pediococcus* sp. (16.7%), *Lactobacillus paracasei* ssp. *paracasei* (22.5%), *L. plantarum* (15.8%), *L. brevis* (9.17%), and *Leuconostoc* sp. (1%). The cheeses' salt content and salt-to-moisture ratio (S/M) were high after 45 days of ripening. The pH values ranged from 5.13 to 5.22. The study highlighted that NaCl and S/M content strongly correlated with the microbial counts. Elevated counts of aerobic mesophilic bacteria, coliform bacteria, and yeasts throughout production and ripening indicated poor sanitary conditions at both farms. This study emphasized the importance of implementing proper hygiene practices throughout cheese production to ensure a high-quality and safe final product. The identified LAB species played a crucial role in the unique flavor of beaten cheese. Furthermore, the study of traditional cheese microflora could help to preserve microbial biodiversity and contribute to the understanding of flavour formation. The prevalence of mesophilic bacteria in both cheese batches was in line with typical findings for traditional cheeses, similar to brined cheeses from the north-eastern Mediterranean countries and the Balkan peninsula (Bintsis and Papademas, 2022).

The study conducted by Dimitrovska et al., (2017) focused on assessing the quality of Beaten cheese. The study examined the microbiological quality of Beaten cheese following a 45-day ripening period. The results showed no detection of harmful *Listeria monocytogenes* and *Salmonella* spp. However, the presence of *Enterobacteriaceae*, which ranged between 20 and 2000 CFU ml^{-1}, could potentially be attributed to the utilization of raw milk in the production of Beaten cheese. It should be noted that cheeses made from raw milk may contain coagulase-positive staphylococci within certain limits, in accordance with the Regulation on microbiological quality. Furthermore, the analysis revealed the presence of *E. coli* within permissible ranges (10 to 1200 CFU ml^{-1}) across all samples. A study done by Talevski, (2012) showed very similar results regarding cow Beaten cheese. Specifically, the presence of *Enterobacteriaceae* in 10 traditionally produced cheeses ranged from 260 to 3000 CFU g^{-1}, *E. coli* ranging from 0 to 1300 CFU g^{-1}, and coagulase-positive *Staphylococcus* from 5 to 110 CFU g^{-1}. Levkov et al., (2019) investigated the yeasts and molds found in traditional ewe's milk beaten cheese from two different farmhouses. Throughout the cheese production and ripening process, yeasts were consistently present. The yeast count peaked during dry salting, brining, and dry ripening stages, with significant species including *Trichosporon pullulans*, *Debariomyces hansenii*, and *Kluyveromyces lactis*. These yeasts are believed to play a crucial role in cheese ripening and in imparting its distinct flavor and aroma. Molds, however, were sporadically present, likely due to environmental contamination, with species such as *Aspergilus niger* and *Fusarium oxysporum* being prevalent. To ensure cheese quality, the study emphasizes the importance of hygiene and sanitation in production areas, as molds indicate contamination from the production environment. Further studies are recommended to explore the properties of these isolated species.

The research conducted by Levkov et al., (2017, 2014) examined the microbial composition of traditional Beaten cheese, revealing the prevalence of mesophilic lactic acid bacteria (LAB) with variations based on the household of production. Among the LAB isolates, *Lactococcus lactis* ssp. *lactis* (35%) dominated the cocci-shaped species. *Pediococcus* genus accounted for 16.7% of isolates, and *Leuconostoc* represented only 1%. *L. lactis* ssp. *lactis* was primarily found in early production stages, not during brine ripening. *Pediococcus* sp. was present throughout ripening stages, while *Leuconostoc* sp. was isolated from curd in one household. *Lactobacilli* species were also identified: *Lactobacillus paracasei* ssp. *paracasei* (22.5%), *L. plantarum* (15.8%), and *L. brevis* (9.2%). *L. paracasei* ssp. *paracasei* and *L. plantarum* were present across curd and ripening stages, while *L. brevis* appeared during dry salting and brine ripening in one household. Due to microbial metabolism potential and the

conditions in which they thrive, the species composition of lactic acid bacteria changes during Beatened cheese production. Among these conditions are the milk properties, the curd formation process, the production process, and the conditions of ripening.

White brine cheese

Traditional production of white brined cheese remains present in Macedonia, and is an economic and important element of rural life. It is produced in small farms and households from raw ewe's milk without addition of starters, using traditional procedures and tools (Levkov et al., 2017). Macedonian white cheese is a brined variety of cheese with either soft or semi- hard texture. White colored, rindless, with close texture and salty acid to pickled flavour are the main sensory properties of this type of cheese. This cheese is most commonly made from cow milk, sheep milk and, less commonly, from goat milk, prepared in blocks, and ripened in brine for a period of 90 days. Traditionally, this type of cheese has been produced for decades by local farmers on a small-scale, using raw milk and traditional techniques handed down from generation to generation using only elementary equipment.

Sheep milk produced by autochthonous sheep breeds is exclusively used for the production of sheep white brined cheese (local sheep breeds, Pramenka, and crossbreeds).

Microbial populations are numerous and diverse. Microorganisms vary in terms of their abundance and diversity during the cheese ripening process. The major microbial groups, isolated from white brined cheese in the first days of the ripening process were *Lactococcus*, *Lactobacillus*, *Enterobacteriaceae*, *Leuconostoc*, *Enterococcus*, and yeasts. As the process evolved, these populations changed and at the end of the ripening process, the most prevalent ones were *Lactobacillus* and *Lactococcus* and yeasts groups (Mojsova et al., 2013).

The study conducted by Levkov et al., (2017) focused on analyzing lactic acid bacteria (LAB) isolated from traditional ewe's milk cheeses, particularly white brined cheese, produced in different regions of North Macedonia. The aim was to examine their phenotypic, physiological, and biochemical characteristics, with a focus on their potential inclusion in future starter cultures. A total of 39 LAB isolates were taken from white brined cheese during both the early (1-week) and late (90-100 days) ripening phases. The dominant species were *Lactobacillus* (59%), *Enterococcus* (23.1%), and *Leuconostoc* (17.9%). Variations were observed in the presence of LAB among different cheese samples and within the same samples during different ripening phases. For instance, *Lb. plantarum*, *Lb. curvatus*, and *Lb. lidneri* were found in the initial week of ripening, while *Lb. plantarum*, *Lb. paracasei* subsp. *paracasei*, and *Lb. brevis* were found in the ripened cheese. Different species of lactobacilli were found in fresh cheese samples from different variants, such as *Lb. plantarum*, *Lb. helveticus*, *Lb. salivarius*, *Lb. curvatus*, and *Lb. delbrueckii* subsp. *delbrueckii*. *Enterococci* were also identified in different cheese samples, including *E. durans, E. faecium, E. fecalis, E. hirae*, and *E. durans*, depending on the region. The study highlighted the importance of genotypic identification alongside phenotypic identification due to discrepancies between the two methods. The isolated LAB species exhibited varying biochemical characteristics, and only specific types of lactobacilli and enterococci demonstrated proteolytic activity. The findings provided valuable insights for designing starter cultures, but further comprehensive investigations into the technological properties of the isolated LAB are necessary for a more complete understanding.

Stojanovski et al. (2021) investigated the Lactobacillus microflora in white cheese made from sheep's milk during a maturation period of 10 to 100 days at a temperature of 10-12°C. A total of 28 strains were isolated from eight samples taken at different stages of ripening and identified as *Lactobacillus* spp. based on growth, gram-stain activity, catalase and oxidase tests, and PCR with genus-specific primers. The study found that *Lactobacillus plantarum* and *Lactobacillus curvatus* were the most common species in the early stages of ripening (10-39 days), while *Lactobacillus plantarum* and *Lactobacillus paracasei* subsp. *paracasei* were

prevalent in later stages (40-80 days). The results suggest that different types of lactobacilli dominate at different stages of white cheese maturation, with *Lactobacillus plantarum* being the dominant species throughout the ripening process.

Kashkaval

The decline in livestock in the Republic of North Macedonia has led to decreased milk production, impacting the overall output of dairy products. This decline is particularly evident in traditional production. As evidence, only a limited number of mountain bachilas (cheese plants up to the mountains near the pastures) are currently involved in cheese production (Santa et al., 2021). One noteworthy traditional cheese is the kashkaval from the Galichnik and Lazaropole regions of the Bistra mountain in North Macedonia. The unique climatic conditions, mountainous terrain, and enduring sheep breeding tradition in the area provide optimal conditions for cheese production. This particular kashkaval, known as Galichki kashkaval and Lazaropolski kashkaval, is traditionally made from raw sheep's milk without the introduction of starter cultures (Santa & Srbinovska, 2014). It is classified under the "pasta filata" category of cheeses, denoting the distinctive plasticization and stretching process characteristic of these cheese types. While there is information available on its physico-chemical properties, the microbiological composition of this cheese remains uncharted.

Sustainability challenges of dairy sector

Lemma et al. (2018) emphasize that as food demands increase through intensive farming, food safety will continue to be a critical issue, particularly for smallholder dairy producers. They point out that intensification, without adequate management, infrastructure, risk analysis, and quality control, can exacerbate food safety issues and pose public health risks. To mitigate these risks, improved management practices, coordinated stakeholder efforts, and the adoption of successful external practices are necessary for sustainable dairy food systems.

Bhat et al. (2022) further highlight the importance of addressing efficiency and sustainability challenges within the global dairy sector. Achieving sustainability requires a state-of-the-art approach, which includes evaluating key sustainability indicators through a holistic framework. Crucial steps include the adoption of green technologies, life cycle analysis, and optimizing the production line. Additionally, the demands of producers, consumers, and the dairy industry must be met while considering future socio-economic and environmental security. The ongoing global climate change crisis has intensified the need to identify responsibilities and find solutions to mitigate its effects on the sector.

In rural areas, the informal dairy sector faces additional challenges, such as inadequate infrastructure, poor transport systems, unreliable electricity supply, substandard hygiene conditions, and insufficient storage and transport facilities. Effective procedures and improved information exchange are also crucial to ensuring product traceability.

In North Macedonia, the sustainability of the dairy subsector is strongly linked to improvements in milk quality and safety. According to the 2020 report by the Food and Veterinary Agency, only 23% of dairy farms meet legal safety standards for milk production, and a mere 2.1% of farms produce raw milk with less than 100,000 bacteria per ml, placing them in the first category. The majority (65%) fall into the second category, with significantly higher bacterial counts. Furthermore, less than 20% of milk purchased meets the quality standards for the extra class, indicating compliance issues with legal regulations. The 2022 Food and Veterinary Agency report reveals that only 8.8% of farms meet the criteria for producing raw milk with a bacterial count below 100,000 per ml, underscoring the need for investments to improve sanitary and hygienic conditions in the sector.

North Macedonian agriculture is largely characterized by small-scale family farms, with cow milk being the primary product used for both direct consumption and processing, and sheep milk mainly for cheese production. However, challenges such as the lack of producer

organizations, underdeveloped cooperatives, and limited access to modern knowledge and technology persist. While efforts have been made towards European Union integration, full alignment with the Common Agricultural Policy (CAP) remains an ongoing goal.

The country is home to various traditional cheeses, which are key to the sustainability of its local food systems. However, local producers face difficulties related to quality, safety, competitiveness, and meeting consumer demands. Many of these producers, although registered as farmers, are not formally recognized as food producers, limiting their ability to seek product protection (Srbinovska & Santa, 2022).

Research into traditional cheeses in North Macedonia underscores the need for greater microbiological understanding. Studies using genomic and metabolomic methods to explore the diversity of artisanal cheeses could provide valuable insights into the microbiological and chemical properties of these products. Additionally, better knowledge of microbial interactions during the cheese production and ripening processes could contribute to improved product safety and sensory quality.

CONCLUSION

Traditional cheese production in the country, such as that of Kashkaval, white brined cheese, and beaten cheese, depends on indigenous lactic acid bacteria, naturally present in raw milk. These microorganisms are critical for the development of the characteristic flavors, textures, and aromas of these cheeses.

The study emphasizes the importance of further investigation into the microbial composition of these cheeses. A deeper understanding of the microbial communities involved could assist in preserving the traditional methods of cheese production and protecting the unique microbial biodiversity inherent to these products. By utilizing modern genomic and metabolomic tools, future research could help to identify the full range of microbial species involved, as well as their interactions during the cheese ripening process.

Furthermore, it is crucial to address the challenges facing small-scale cheese producers, particularly in relation to milk quality and safety standards. Investments in improved hygiene practices and technologies will support the sustainability of traditional cheese production. Collaborative efforts among researchers, producers, and policymakers are needed to promote these traditional products, ensuring their quality and safety while safeguarding their unique cultural and microbial heritage for future generations.

In summary, a comprehensive approach to research, innovation, and education is necessary to maintain the balance between tradition and progress in the North Macedonian dairy sector, while also ensuring the long-term preservation of its microbial diversity and artisanal practices.

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