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Original Scientific Paper

Production techniques and microbial stability of traditional pasta filata cheese throughout its shelf life

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ABSTRACT

Traditional rolled *pasta filata* cheese is a distinctive product valued for its elastic texture, mild flavour and handcrafted appearance. This research aims to show the traditional production technique of rolled *pasta filata* cheese and assess the evolution of its microbial stability throughout shelf life. The cheese is typically produced by stretching the curd in hot water, followed by manual rolling and shaping. During storage, significant changes occur due to moisture migration, proteolysis, lipolysis, and microbial activity. This paper summarizes findings on microbial changes during cold storage under vacuum packaging. Understanding the behaviour of traditional rolled *pasta filata* cheese during its shelf life is essential for maintaining product quality, safety and authenticity, especially as consumer demand for artisanal and minimally processed dairy products grows. The future research can contribute to the preservation and valorisation of traditional cheesemaking heritage, while meeting modern consumer demands for natural, authentic and high-quality dairy products.

1. Introduction

Rolled cheeses are traditionally produced in Vojvodina, the northernmost part of Serbia, and represent *pasta filata* cheeses, which are mainly made from raw cow's milk, raw sheep's milk, or a combination of those two. The production process of rolled cheeses is specific and requires knowledge and skills that are passed down through generations (*Popović-Vranješ et al*., 2017; *Vidaković Knežević et al*., 2024).

The production of rolled cheeses typically begins with the spontaneous fermentation of raw milk. Once the milk reaches a certain level of acidity, a small amount of coagulating enzyme is added. The formed curd is then cut, processed, and the whey is separated. After that, the curd is thermally treated in warm whey or brine, resulting in a stretchable cheese mass that is hand-stretched, salted, and shaped into rolls (*Popović-Vranješ et al.*, 2017). In addition to salt, creative producers in Vojvodina often use various spices and additives, such as paprika, pepper, oregano, basil, chives, sesame, coconut, nuts and chocolate, to enhance the flavour of rolled cheese.

Rolled cheeses are characterized by the regular shape of the rolls. The texture is smooth and elastic, while the consistency is usually semi-hard. The cross section is layered. The colour of rolled cheese is white with yellowish shades. Rolled cheese has a milky, not too sour or salty taste, and a slight milky-sour smell (*Vidaković Knežević et al*., 2024).

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These types of cheeses are complex microbial ecosystems. The sources of microorganisms in rolled cheeses are diverse. Primarily, they include the microbiota of raw milk and of the surfaces and hands that encounter the cheese.

The natural microbiota of raw milk is heterogeneous in composition, but is mainly rich in lactic acid bacteria, such as *Lactococcus* spp., *Streptococcus* spp., *Lactobacillus* spp., *Leuconostoc* spp., and *Enterococcus* spp., which enable the spontaneous fermentation of milk and contribute to the flavour development of the final product (*Quigley et al*., 2013). In addition to these bacteria, raw milk also contains various representatives of both Gram-positive (*Bacillus, Clostridium, Staphylococcus, Micrococcus, Microbacterium, Corynebacterium*) and Gram-negative (*Pseudomonas, Flavobacterium, Acinetobacter, Aeromonas, Enterobacteriaceae*) bacteria (*Tilocca et al*., 2020). Additionally, many yeasts and moulds can contribute to the flavour, aroma and texture of some types of cheese. However, in the case of rolled cheeses, they can be the cause of spoilage and the development of undesirable sensory characteristics (*Kure and Skaar*, 2019).

Coagulase-positive staphylococci, *Enterobacteriaceae* and *Escherichia coli* are highlighted as process hygiene criteria in the raw milk cheese production process, while *Listeria monocytogenes* and *Salmonella* spp. are considered food safety criteria (*Official Gazette of the Republic of Serbia*, 2024). Since rolled cheese is made from raw milk and belongs to the category of fresh *pasta filata* cheeses (*Official Gazette of the Republic of Serbia*, 2014), it has a short shelf life.

The objectives of this research were to present the production technology of traditional rolled cheese and to determine its microbiological quality during cold storage under vacuum packaging.

2. Materials and methods

2.1. Cheese production

Raw cow's milk was left to spontaneously ferment using its natural microbial population at 20 °C for 24 hours. The resulting curd was cut and then separated from the whey by the action of its own weight through a cotton cloth. After the curd draining process was completed, the curd was steamed in water at 70 °C to 80 °C, with constant mechanical stretching using a wooden spoon. Once the cheese dough reached a plastic consistency, further kneading, shaping, and stretching were performed by hand on the table. The warm dough was stretched to 5 mm thickness and by folding the stretched cheese dough inwards, the required width was achieved. At this stage, the cheese dough was hand salted. To form rolls of approximately equal size, cheese dough was then rolled inwards from both sides. The cheese rolls were then wrapped in cotton cloth and stored at 4 °C for 24 hours to allow the excess whey to drain. Then, the rolled cheeses were vacuum-packed and stored at 4 °C.

2.2. Microbiological analysis

Following the reference methods, total viable count (TVC) were counted on plate count agar (PCA, CM0325, Oxoid, United Kingdom) according to the SRPS EN ISO 4833-1 (*ISO*, 2014); *Enterobacteriaceae* were counted on violet red bile glucose (VRBGA, CM1082, Oxoid, United Kingdom) according to the SRPS EN ISO 21528-2 (ISO, 2017a); *Escherichia coli* were counted on tryptone bile X-glucuronide medium (TBX, CM0945, Oxoid, United Kingdom) according to the SRPS ISO 16649-2 (*ISO*, 2008); coagulase-positive staphylococci were counted on Baird-Parker agar (Biokar Diagnostics, France) according to the SRPS EN ISO 6888-1 (*ISO*, 2021); *Salmonella* spp. were detected according to SRPS EN ISO 6579-1 (*ISO*, 2020); and *Listeria monocytogenes* were detected according to SRPS EN ISO 11290-1 (*ISO*, 2017b).

2.3. Statistical analysis

Data were analysed using R statistical software (version 3.2.2; R Foundation for Statistical Computing, Vienna, Austria). One-way analysis of variance (ANOVA), followed by Duncan`s multiple range test, was performed to evaluate differences in microbial populations, with a significant level set at $p < 0.05$.

3. Results and discussion

The microbial status of the rolled cheese is presented in Table 1. During storage of rolled cheese at 4°C over 60 days, there was a significant ($p < 0.05$) decrease in the number of aerobic mesophilic bacteria by 0.28 $\log_{10}$ CFU/g, *Enterobacteriaceae* by 1.34 $\log_{10}$CFU/g, and *Escherichia coli* by 1.97 $\log_{10}$ CFU/g. The only increase was observed in the number of coagulase-positive staphylococci, which rose by 0.7 $\log_{10}$ CFU/g.

Table 1. Microbial status of traditionally produced rolled *pasta filata* cheese vacuum-packed and stored at 4 °C over 60 days

Microorganisms	Day 0	Day 20	Day 40	Day 60
Aerobic mesophilic bacteria (log_{10} CFU/g)	8.03 ± 0.11^{a}	7.89 ± 0.11^{ab}	7.35 ± 0.19^{c}	7.75 ± 0.21^{b}
Enterobacteriaceae (log_{10} CFU/g)	2.87 ± 0.16^{a}	3.06 ± 0.57^{a}	1.81 ± 0.16^{b}	1.53 ± 0.61^{b}
Escherichia coli (log_{10} CFU/g)	3.12 ± 0.47^{a}	2.42 ± 0.11^{b}	1.32 ± 0.23^{c}	1.15 ± 0.17^{c}
Coagulase-positive staphylococci (log_{10} CFU/g)	1.40 ± 0.61^{b}	2.28 ± 0.18^{ab}	2.83 ± 0.43^{a}	2.10 ± 0.83^{ab}
Salmonella spp. (in 25 g)	Not detected	Not detected	Not detected	Not detected
Listeria monocytogenes (in 25 g)	Not detected	Not detected	Not detected	Not detected

Legend: Values within the same row marked with different superscript letters ($^{a, b, c}$) indicate statistically significant differences ($p < 0.05$).

Compared with the current microbial counts, our previous research showed lower counts of *Enterobacteriaceae*, *Escherichia coli* and coagulase-positive staphylococci in rolled cheeses made with cow's and donkey's milk (*Vidaković Knežević et al.*, 2025). This could be attributed to the slightly different production method of the rolled cheeses, as previously, the milk was heated to 40 °C before the addition of rennet.

The aerobic mesophilic bacteria can be used as part of a general assessment of food quality during its shelf life. Aerobic mesophilic bacteria levels below 6 log_{10} CFU/g indicate a diverse microbiota, while numbers above this level indicate the dominance of a particular microorganism which, depending on the type of food, could affect the organoleptic properties of the food. Although aerobic mesophilic bacteria levels above 7-8 log_{10} CFU/g are often considered as indicators of unsatisfactory microbiological quality, this level cannot be generalized. In the case of fermented dairy and cheese products, aerobic mesophilic bacteria are not suitable as quality indicators. For products containing high levels of these bacteria, additional testing should be carried out to determine the number of yeasts, Gram-negative bacteria and lactic acid bacteria (if they are not intentionally added during the manufacturing process). In the case of rolled cheese, the product is not satisfactory if it contains >6 log_{10} CFU/g of yeasts, >7log_{10} CFU/g Gram-negative bacteria and >8 log_{10} CFU/g lactic acid bacteria (*Health Protection Agency*, 2009).

Escherichia coli is a Gram-negative, rod-shaped bacterium that belongs to the coliform bacteria, a group of bacteria in the family *Enterobacteriaceae* (*International Dairy Federation*, 2016). Ruminants excrete these bacteria through their faeces, which via milking teats can then contaminate the udder and, thus, the milk. A previous study showed a high contamination rate of *Escherichia coli* in traditionally-produced cheeses in the Banat region of Romania (*Imre et al.*, 2022). In fermented dairy products, however, relatively low numbers of *Enterobacteriaceae* and *Escherichia coli* can be due to the antagonistic activity of lactic acid bacteria. It is believed that lactic acid bacteria prevent the multiplication of bacteria from the *Enterobacteriaceae* family, which includes *Escherichia coli*, through several control mechanisms. Firstly, these two groups of bacteria (lactic acid bacteria and *Enterobacteriaceae*) compete for nutrients. Additionally, during the fermentation process, lactic acid bacteria produce acids that are considered potential inhibitors of *Enterobacteriaceae* (*Mladenović et al.*, 2021).

Subclinical staphylococcal mastitis is a common source of the coagulase-positive staphylococci (*Kousta et al.*, 2010). Some strains of coagulase-positive staphylococci can produce enterotoxins, which are heat-stable proteins. The ingestion of enterotoxins through food leads to foodborne intoxication, manifested by vomiting and diarrhoea (*Katić*, 2008). The prevalence of coagulase-positive staphylococci varies among different types of cheese, and up to 80% of raw milk cheeses contain these bacteria (*Johler et al.*, 2018). The slight increase in coagulase-positive staphylococci in the rolled cheeses during storage in the current study was likely the result of an ideal environment, namely a high content of proteins and fats, a relatively acidic medium and low salt content (*Al-Nabulsi et al.*, 2020).

In this study, neither *Salmonella* or *Listeria monocytogenes* were detected in the rolled cheeses. Previously, *Salmonella* Typhimurium, *Salmonella* Enteritidis, *Salmonella* Dublin and *Salmonella* Montevideo were detected in cheeses made with raw milk (*Yoon et al*., 2016). Also, *Listeria monocytogenes* was detected in up to 37.5% of fresh cheeses, in up to 55% of soft and semi-hard cheeses, and in up to 5.5% of hard and extra-hard cheeses (*Gérard et al*., 2018).

4. Conclusion

Traditional rolled *pasta filata* cheese is distinctive artisanal product, appreciated for its texture, mild flavour, and handcrafted appearance. This study presented the traditional production method and examined microbial changes during refrigerated storage under vacuum packaging. The results showed a general decline in the populations of aerobic mesophilic bacteria, *Enterobacteriaceae* and *Escherichia coli* during storage, while a slight increase was observed in the number of coagulase-positive staphylococci.

Understanding these microbial dynamics is essential for ensuring the safety, quality and authenticity of traditional dairy products. Preserving and promoting traditional cheesemaking practices, alongside meeting modern consumer preferences for natural and minimally processed foods, remains an important direction for future research and development.

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