PhD THESIS

The quality and typicity of Feta cheese

SUMMARY OF THE PhD THESIS

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INTRODUCTION

In the context of globalized food markets, ensuring the quality, authenticity, and microbiological safety of dairy products is essential for consumers, producers, and authorities. Traditional cheeses, such as Feta and its derivatives, hold significant cultural and economic value in Europe and are protected by regulations such as the Protected Designation of Origin (PDO). However, risks of counterfeiting and adulteration, especially through the use of unauthorized types of milk, underline the need for modern authentication methods.

Buffalo milk, known for its high nutritional value, was analyzed as a potential alternative for diversifying Romanian Feta-type cheese production. Simultaneously, ensuring the microbiological safety of dairy products, particularly against Listeria monocytogenes contamination, remains a priority amid increasing bacterial resistance to antimicrobial treatments.

This thesis proposes an integrated approach to dairy product quality, authenticity, and food safety, with three main objectives:

1. Evaluating the quality of raw buffalo milk for Feta-type cheese production.
2. Authenticating Feta and Feta-type cheeses from Romania and Greece using advanced multi-analytical methods.
3. Assessing the prevalence and antimicrobial resistance profiles of Listeria monocytogenes strains isolated from dairy products marketed in Romania.

By combining traditional and modern methods, the thesis offers practical solutions for improving dairy quality and safety standards within the European context.

THE STRUCTURE OF THE PhD THESIS

The PhD thesis entitled *"The quality and typicity of Feta cheese"* consists of 109 pages and includes 16 figures and 7 tables. It is structured into two main parts and has been developed according to the editorial standards of IOSUD USAMV-Cluj-Napoca.

The first part, comprising 24 pages and 3 chapters: **Chapter 1**: *"Feta and Feta-Type Cheese: Definition, Characteristics, and Regulations"*, discusses the origin, features, and PDO status of Feta cheese, and differences between authentic Feta and Feta-type cheeses produced outside Greece. **Chapter 2**: *"Production, Composition, and Quality of Feta and Feta-Type Cheese"*, covers traditional and industrial production methods, chemical composition, and factors influencing cheese quality. **Chapter 3**: *"Food Authenticity Assessment"*, presents modern authentication techniques, including isotope ratio mass spectrometry (IRMS), elemental analysis (ICP-MS), and PCR molecular detection.

The second part spans 51 pages and contains 6 chapters, presenting hypotheses, objectives, materials, methods, results, discussions, and conclusions of the three original

studies conducted. The thesis concludes with general conclusions and originality and innovative contributions.

THE OBJECTIVES OF THE PhD THESIS

The general objective of the thesis was the complex evaluation of the quality, authenticity, and safety of Feta and Feta-type cheeses using modern analytical methods. Three specific objectives were formulated:

- **Specific Objective 1**: Dynamic evaluation of raw buffalo milk quality parameters using high-performance equipment;
- **Specific Objective 2**: Authentication of Feta and Feta-type cheese samples from Romania and Greece by integrating isotope ratio mass spectrometry, elemental analysis, and PCR techniques;
- **Specific Objective 3**: Assessment of the prevalence and antimicrobial resistance profiles of Listeria monocytogenes strains isolated from dairy products marketed in Romania.

Study 1. Dynamic Evaluation of Quality Parameters of Raw Buffalo Milk

Introduction

Buffalo milk occupies an important place in the global dairy production landscape, being the second most consumed type of milk after cow's milk. It contains higher levels of fat, proteins, and minerals compared to cow's milk, making it extremely valuable for producing premium dairy products such as Feta-type cheeses. Despite its nutritional and economic potential, buffalo milk remains commercially underexploited. Milk quality is influenced by a range of factors, including seasonality, milking hygiene, animal health, and the technologies used for collection and processing. Monitoring the physicochemical and microbiological parameters of raw milk is crucial for assessing its technological potential and for preventing microbiological contamination. Modern technologies, such as CombiFoss™ analyzers, allow rapid and precise quality assessment and are valuable tools in quality control within dairy farms.

Aim of the study

The main objective of the study was to determine the physicochemical and microbiological composition of raw buffalo milk throughout a one-year period and to evaluate the influence of seasonality, hygiene practices, and the performance of the CombiFoss™ 7 equipment.

Materials and Methods

Twenty-four samples of raw buffalo milk were collected biweekly from March 2020 to February 2021 from a certified farm housing 500 buffaloes, 190 of which were lactating. The milking process was mechanized, and samples were taken directly from the cooling tanks, transported under refrigerated conditions, and analyzed using the CombiFoss™ 7 system, according to international standards.

Results and discussions

The mean fat content was 8.821 g/100 g, slightly higher than commonly reported international values. The average protein content was 4.400 g/100 g, showing minimal seasonal variation, while lactose and total solids content remained consistent, with slight decreases during the summer attributed to diet changes. The pH values remained stable between 6.61 and 6.9, suggesting good udder health.

Urea content displayed seasonal variability, with lower levels during the summer grazing period.

The somatic cell count (SCC) had an annual mean of 304.85×10^3 cells/mL, with occasional exceedance of the European limit in April and February.

The total viable count (TVC) showed a high annual average of 2059.65×10^3 CFU/mL, exceeding European standards in more than half of the samples, indicating the need for improved milking hygiene, particularly during the winter.

Conclusions

Buffalo milk showed excellent nutritional composition but revealed hygiene challenges during certain periods, especially when animals were kept indoors. Enhancing hygiene practices during milking, collection, and transport is essential to ensure superior microbiological quality.

Study 2 – Authentication of Feta and Feta-Type Cheese Samples from Romania and Greece Using IRMS, Elemental Analysis, and PCR Techniques

Introduction

Ensuring the authenticity and geographical origin of dairy products, particularly traditional cheeses, has become a priority in the context of quality protection and food fraud prevention. Feta cheese, certified as a Protected Designation of Origin (PDO) product in 2002, is a key example, produced under strict traditional rules in specific Greek regions.

Aim of the study

The main aim was to develop and apply an integrated method combining IRMS, elemental profiling, and PCR techniques to evaluate the authenticity of dairy products, particularly Feta and Feta-type cheeses from Greece and Romania, by detecting potential species substitution and verifying their geographical origin.

Materials and Methods

Thirteen samples of raw milk and cheeses were collected from markets in Greece and Romania between 2021 and 2023. Species identification was performed through PCR amplification of the mitochondrial cytochrome B gene, while isotopic and elemental profiles were determined using IRMS and ICP-MS. Statistical analysis included PCA for exploring natural variations and PLS-DA for precise sample classification by origin and type.

Results and discussions

PCR analysis confirmed the species composition of authentic Greek Feta, detecting only sheep and goat DNA as expected. Several Romanian Feta-type cheese samples, however, revealed undeclared cow's milk content, indicating possible fraudulent practices. Additionally, one sample labeled as buffalo milk was identified as cow's milk.

Isotopic and elemental analyses highlighted clear separations between Greek Feta and Romanian Feta-type cheeses, with significant differences in elements such as Sn, Li, Mn, K, and $\delta^{13}C$ values. PLS-DA analysis achieved 100% classification accuracy based on

geographical origin and product type, demonstrating the effectiveness of the multi-analytical approach.

Conclusions

The integration of IRMS, elemental analysis, and PCR proved to be highly effective for dairy product authentication. Despite the limited number of samples, the results support the broader application of this methodology in routine food authenticity controls and regulatory inspections.

Study 3. Assessment of the Prevalence and Antimicrobial Resistance Profile of Listeria monocytogenes Strains Isolated from Dairy Products Marketed in Romania

Introduction

Listeria monocytogenes (*L. monocytogenes*) is a major foodborne pathogen, frequently linked to severe infections resulting from the consumption of contaminated dairy products, especially ready-to-eat foods. Its remarkable ability to survive under environmental stress conditions makes it a persistent threat throughout the food production chain.

Aim of the study

The aim was to evaluate the prevalence of *L. monocytogenes* in dairy products marketed in Romania over a three-year period (2021–2023), and to characterize the antimicrobial resistance profiles and molecular serotypes of the isolates.

Materials and Methods

A total of 10,306 dairy samples, including milk, cheese, yogurt, cream, and ice cream, were analyzed. *L. monocytogenes* isolation followed international standards, with biochemical confirmation and molecular serotyping through multiplex PCR. Antimicrobial susceptibility testing was performed according to EUCAST and CLSI

guidelines. Data were analyzed statistically using Python and visualized with graphs and charts.

Results and discussions

Out of 10,306 dairy samples, 43 isolates of *L. monocytogenes* were recovered, resulting in an overall prevalence of 0.41%. The majority of positive cases were found in ice cream and fresh cow's milk cheeses. Products with unspecified or minimally applied thermal treatments showed higher contamination rates.

Molecular serotyping revealed a predominance of serogroup IVb, followed by IIa and IIb, patterns that are consistent with international trends where Lineage I strains are often linked to clinical cases and outbreaks. Antimicrobial resistance testing showed the highest resistance rates against oxacillin and trimethoprim-sulfamethoxazole, while no resistance to fluoroquinolones was detected.

Conclusions

Although the overall prevalence of contamination was low, the presence of pathogenic serogroups and antimicrobial-resistant strains highlights the importance of continuous monitoring and strict hygienic control, particularly in ready-to-eat dairy products. Further research involving detailed genetic characterization of isolates is recommended.

GENERAL CONCLUSIONS

This doctoral thesis comprehensively evaluated the quality, authenticity, and microbiological safety of dairy products, with a particular focus on Feta and Feta-type cheeses, through the application of advanced, integrated analytical methods.

The first study:

- demonstrated that raw buffalo milk has a high-fat content and stable physicochemical characteristics, making it a valuable raw material for producing high-quality dairy products;
- the observed variations in microbiological parameters, correlated with seasonality and hygiene practices, highlight the urgent need to improve milking and milk handling hygiene, especially during the winter period when the animals are housed indoors.

The second study:

- validated the effectiveness of combining IRMS, ICP-MS, and PCR techniques for authenticating Feta and Feta-type cheeses;
- clear differentiation between authentic Greek Feta and Romanian Feta-type cheeses was achieved, and cases of food fraud through milk species substitution were identified;
- the integrated multi-analytical method proposed in this study proved to be a powerful tool for routine food authenticity control and could significantly enhance the reliability of quality certification processes.

The third study:

- assessed the prevalence and antimicrobial resistance of *L. monocytogenes* strains isolated from dairy products marketed in Romania;
- although the overall prevalence was low (0.41%), the dominant presence of serogroup IVb, known for its association with clinical cases, raises significant concerns;
- notable levels of antimicrobial resistance, particularly to oxacillin, ampicillin, tetracycline, and trimethoprim-sulfamethoxazole, were detected, underscoring the importance of continuous monitoring and targeted safety measures.

Overall, the results obtained from this research support the integration of modern authentication and microbiological control methods into the dairy sector, emphasizing the necessity for rigorous policies on hygiene, food safety, and the protection of authentic products. The work contributes meaningfully to the advancement of scientific knowledge in the field, offering practical solutions for improving food quality and safety standards in the European context.

ORIGINALITY AND INNOVATIVE CONTRIBUTIONS OF THE THESIS

The originality of this thesis lies in the integration of isotopic, elemental, and molecular methods for the authentication of Feta and Feta-type cheeses, representing the first application of such a complex approach in Romania. The study on buffalo milk provided new data through the use of the CombiFoss™ equipment, while the analysis of *Listeria monocytogenes* offered a detailed overview of the prevalence and antimicrobial resistance in ready-to-eat dairy products. Through the proposed methods and the results obtained, the thesis contributes to the development of a modern platform

for monitoring food quality and safety, supporting the protection of traditional products and the fight against food fraud.