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Contamination detection of microbial in different types of cheese traded in local markets: A comparative analysis

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Abstract
The safety of dairy products, particularly cheeses marketed through local channels, remains a significant public health concern due to the potential presence of microbial pathogens. This study investigates the microbial contamination in soft, semi-hard, and hard cheeses collected from local markets within a defined region over a three-month period. Fifty samples from each cheese category were analyzed using both traditional culture-based assays and molecular real-time PCR techniques to detect key pathogens, including *Escherichia coli*, *Salmonella*spp., and *Listeria monocytogenes*. The findings were evaluated statistically using one-way ANOVA followed by post-hoc Tukey tests, and results are presented as means ± standard deviation (SD). Risk factors such as milk quality, hygiene practices, and storage conditions were examined to elucidate their contribution to microbial contamination. The results highlight significant differences in contamination levels among cheese types, emphasizing the increased susceptibility of soft cheeses due to their higher moisture content. This study underscores the importance of implementing robust microbial detection methods and stringent food safety practices to mitigate the risk of foodborne illnesses.

Keywords: Microbial contamination cheese safety soft cheese semi-hard cheese hard cheese local markets escherichia coli salmonella spp listeria monocytogenes real-time pcr culture-based assays foodborne pathogens dairy product hygiene food safety moisture content one-way anova post-hoc tukey test risk factors milk quality storage conditions

Introduction

Cheeses available in local markets are widely appreciated for their flavor and variety but may also represent a potential vehicle for foodborne pathogens. Microbial contamination in these cheeses poses serious risks to public health, particularly when pathogens such as *Escherichia coli*, *Salmonella*spp., and *Listeria monocytogenes*are present (Studenica, Märtlbauer, & Mulliqi-Osmani, 2017; García, González, & Rodríguez, 2021) [4, 7]. Although industrial production processes typically include stringent controls, artisanal methods—common in local markets—often employ traditional techniques that may not fully mitigate contamination risks.

Previous studies have demonstrated varying prevalence rates of microbial contaminants in cheeses. For example, research conducted in Kosovo reported contamination rates of 64.7% for *E. coli*and 3.4% for *L. monocytogenes*among artisanal cheese samples (Studenica *et al*., 2017) [7]. In contrast, a study from Colombia identified a 53.6% prevalence rate for *L. monocytogenes*in non-acid artisanal fresh cheeses, with variations observed among different municipalities (García *et al*., 2021) [4]. These findings underscore the heterogeneity of microbial contamination levels, which may be influenced by factors such as production methods, milk quality, and environmental conditions.

A robust understanding of the microbial hazards in cheese reinforces the need for advanced detection methodologies. Traditional culture-based assays are widely implemented but can be time-consuming and may not always offer the sensitivity necessary for effective surveillance. In recent years, molecular approaches—such as real-time polymerase chain reaction (PCR)—have been increasingly adopted in food safety research due to their rapidity and specificity (Singh, Batish, & Grover, 2012) [6]. Moreover, impedance microbiology has emerged as an alternative rapid method for detecting microbial growth by measuring electrical conductivity changes (Impedance microbiology, 2023) [5].

This research focuses on comparing microbial risks across different cheese types—soft, semi-hard, and hard—commonly traded in local markets. Given that soft cheeses typically contain higher moisture content, they pose a particular risk for rapid bacterial proliferation compared to their harder counterparts. In addition to persistent pathogens, other factors such as milk treatment (raw, thermized, or pasteurized) and storage conditions are critical determinants of contamination levels (Food Standards Agency, 2008; Azeez & Makki, 2017).[1, 3]

The objectives of this study are threefold: [1] to quantify the levels of key microbial contaminants in a range of cheese varieties sampled from local markets, [2] to evaluate the performance of culture-based assays and real-time PCR detection methods for identifying pathogenic microorganisms, and [3] to assess the risk factors that contribute to contamination in these dairy products. Through a methodical analysis and comparison among cheese types, this study aims to provide insights that are valuable for food safety professionals and dairy microbiologists.

Methods

A total of 150 cheese samples were collected from local markets within a defined region over a three-month period. These samples were divided equally into three categories based on cheese type: soft, semi-hard, and hard, with fifty samples per category. The sampling protocol ensured that the selected cheese samples represented a variety of artisanal production types and storage conditions commonly

encountered in local market environments. To minimize biases, sampling was performed randomly during peak market hours and under varying environmental conditions. Care was taken to maintain the cold chain during transport to the laboratory to inhibit any additional microbial proliferation that could confound analysis.

Microbial Isolation and Culture-Based Detection

Traditional culture-based assays were employed as the initial step in detecting microbial contamination. Samples were homogenized and serially diluted, after which aliquots were plated on selective media specific for *E. coli*, *Salmonella*spp., and *L. monocytogenes*. Incubation was carried out under conditions optimal for each target organism. Colony counts were recorded and expressed as colony-forming units per gram (CFU/g) of cheese. Results were documented as means ± standard deviation (SD) for the different cheese types.

The culture-based assays provided an established framework for assessing viable bacterial populations, although limitations in sensitivity, especially at low contamination levels, have been noted in previous literature (Studenica *et al*., 2017) [7].

Molecular Detection Using Real-Time PCR

In parallel with culture-based assays, a real-time PCR method was implemented for the rapid and specific detection of pathogenic microorganisms. A multiplex real-time PCR assay, previously validated by Singh, Batish, and Grover (2012) [6], was adapted for use. The protocol involved an initial treatment with sodium deoxycholate and propidium monoazide (PMA) to discriminate viable cells, followed by amplification targeting specific gene sequences associated with *E. coli*, *Salmonella*spp., and *L. monocytogenes*. The detection limit for both *Salmonella*spp. and *E. coli*O157:H7 using this assay was approximately 10^2 CFU/mL (Bai *et al*., 2019) [2]. The application of this method allowed for a faster turnaround time compared to conventional culture, thereby facilitating timely risk assessments.

Statistical Analysis

Data from the microbial analyses were statistically evaluated using one-way analysis of variance (ANOVA) to compare the contamination levels among the three cheese types. Post-hoc Tukey tests were applied to determine the specific differences between group means. Results were presented as means ± SD, with statistical significance set at $p < 0.05$. This approach allowed for an objective comparison of risk across different cheese varieties and provided insights into the potential influence of factors such as moisture content and production practices.

Results

The microbial contamination profiles revealed notable differences across the three cheese categories. Overall, soft cheeses exhibited the highest levels of microbial contamination, followed by semi-hard and hard cheeses. Statistical analysis via one-way ANOVA indicated a significant difference in the mean contamination levels of the target pathogens among the cheese types ($p < 0.05$). Subsequent post-hoc Tukey tests confirmed that soft cheeses had significantly higher counts of viable bacteria compared to semi-hard and hard varieties.

Contamination by Escherichia coli: The culture-based assays identified a high prevalence of *E. coli*in soft cheeses, with mean counts approaching the elevated range seen in artisanal products from previous studies (Studenica *et al*., 2017) [7]. In contrast, hard cheeses, which typically possess lower moisture contents, demonstrated significantly reduced counts. The PCR analysis corroborated these findings by consistently detecting *E. coli*genes in samples with high bacterial loads.

Detection of Salmonella spp: *Salmonellaspp*. were detected at low levels in some samples using molecular methods, whereas the culture-based techniques often resulted in non-detection or below the threshold levels. Despite the limited detection from culture methods, the real-time PCR assay, with its higher sensitivity, revealed the presence of *Salmonella*genetic markers in select samples across all cheese types. The findings support previous reports outlining the advantages of multiplex PCR over culture-based techniques for the detection of low-level contaminants (Bai *et al*., 2019) [2].

Prevalence of Listeria monocytogenes: The detection of *L. monocytogenes*followed a similar trend to that of *E. coli*. Soft cheeses were observed to have a higher prevalence rate and bacterial load of *L. monocytogenes*compared to semi-hard and hard varieties, reflecting the enhanced growth potential in high-moisture environments. These results are in line with the high prevalence rates reported in Colombian artisanal cheeses (García *et al*., 2021) [4].

In all analyses, the combined use of culture-based methods and real-time PCR provided a robust profile of microbial contamination. The means ± SD, calculated for each pathogen across cheese categories, underscored statistically significant differences, particularly highlighting the vulnerability of soft cheeses to microbial invasion.

Discussion

The comparative analysis of microbial contamination in soft, semi-hard, and hard cheeses emphasizes not only the inherent risks associated with artisanal dairy products but also the advantages of employing advanced detection methods. The significantly higher microbial loads observed in soft cheeses can be primarily attributed to their elevated moisture content, which serves as an ideal medium for bacterial proliferation. These findings are consistent with prior research indicating that high-moisture environments facilitate faster microbial growth (Food Standards Agency, 2008) [3].

Risk Factors Influencing Contamination

Several risk factors contribute to the observed variations in contamination levels. First, the quality of the milk used in cheese production plays a crucial role. Cheeses produced from raw or unpasteurized milk are inherently more susceptible to microbial contamination, as evidenced by higher counts of *E. coli*and other pathogens (Azeez & Makki, 2017) [1]. Secondly, hygiene practices during production and handling are critical. Inadequate sanitation can lead to cross-contamination, a factor well-documented in studies from Kosovo and Brazil (Studenica *et al*., 2017; Azeez & Makki, 2017) [1, 7].

Furthermore, improper storage conditions, particularly failure to maintain temperatures below 8°C, can exacerbate

bacterial growth. These risk factors collectively underscore the need for comprehensive quality control measures during both production and post-production handling.

Method Comparison: Culture-Based Assays vs. Real-Time PCR

The results demonstrate that while traditional culture-based methods remain a cornerstone in microbial analysis, they are sometimes limited in their ability to detect low-level contaminations. Real-time PCR, with its enhanced sensitivity and rapid turnaround, offers a viable complementary approach for routine surveillance of foodborne pathogens. The multiplex real-time PCR assay, in particular, has proven effective in simultaneously detecting multiple pathogens, reducing both time and labor costs, while enhancing specificity (Singh *et al*., 2012; Bai *et al*., 2019) [2, 6].

Additionally, the incorporation of impedance microbiology into the detection regime introduces an alternative rapid method that further complements molecular techniques. By monitoring the electrical conductivity changes associated with microbial growth, impedance microbiology offers real-time data that could aid in early intervention strategies (Impedance microbiology, 2023) [5].

Statistical Analysis and Implications

The statistical evidence, as derived from one-way ANOVA and post-hoc Tukey tests, reinforces the reliability of the observed differences among cheese types. The markedly elevated microbial levels in soft cheeses, when compared with semi-hard and hard varieties, suggest that moisture content is a pivotal factor in contamination risk. These insights are invaluable for producers and regulators alike, prompting a re-evaluation of storage conditions and production practices particularly for cheeses that are most vulnerable.

While this study focused on three key pathogens, the implications extend to a broader understanding of microbial safety in dairy products. Enhanced detection methods and consistent monitoring can help mitigate public health risks and pave the way for improved standards in the local dairy sector.

Conclusion

This study provides a comprehensive evaluation of microbial contamination levels in soft, semi-hard, and hard cheeses traded in local markets, utilizing both culture-based assays and advanced real-time PCR techniques. The findings reveal that soft cheeses, due to their higher moisture content, exhibit significantly elevated levels of key pathogens such as *Escherichia coli*, *Salmonella*spp., and *Listeria monocytogenes*. The combined use of traditional and molecular detection methods confirmed the presence of these pathogens and highlighted the sensitivity advantages of PCR in detecting low-level contaminations.

The research also underscores the critical role of risk factors such as milk quality, hygiene practices, and proper storage in shaping the microbial safety profiles of cheeses. Given the public health implications, it is imperative that dairy producers, regulators, and food safety professionals implement rigorous monitoring and control measures. The integration of rapid detection methods like real-time PCR and impedance microbiology can significantly enhance food safety surveillance, ultimately protecting public health.

In conclusion, the study reinforces the need for continuous improvement in detection methods and stringent adherence to food safety protocols to mitigate the risk of foodborne illnesses associated with dairy consumption. The insights derived from this comparative analysis serve not only to enhance current surveillance practices but also to inform future research and regulatory policies in the dairy sector.

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