



An Evaluation of Microbial Contamination in Market Cheese

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ABSTRACT:

The diverse world of cheese owes its existence, in no small part, to the microscopic maestros known as microorganisms. These culinary collaborators play a crucial role in crafting the distinct flavors, textures, and aromas that set each cheese variety apart. But beyond their delicious contributions, understanding the microbial landscape of cheese is vital for ensuring its safety and quality. This study delves into the fascinating interplay between microbes and five distinct cheese types. By focusing on the tango between total bacteria count, Lactic Acid Bacteria (LAB) count, and the enigmatic presence of fungi, the researchers shed light on the microbial composition of each cheese. The study found significantly higher bacterial levels in traditional Chinese cheese and soft cheese compared to their firmer counterparts. The study went beyond simply counting microbes; it ventured into the realm of potential health hazards. By analyzing the colony forming units (CFU/ml) – a measure of microbial concentration – collected from various sources, we assessed the likelihood of hazard, using machine learning statistics, associated with each cheese type. Their findings revealed a concerning correlation: as the total bacteria and fungi counts increased, so did the potential health risk. The high colony forming total bacteria and fungi, coupled with the elevated hazard likelihood, highlight the need for stricter quality control measures for these cheese varieties.

Introduction

The cheese is a complex microbial ecosystem that contains a diverse array of bacteria, yeasts, and molds (Zheng et al., 2021). The microbial communities in cheese can vary depending on various factors, such as cheese type, milk source, processing methods, and ripening conditions (Zheng et al., 2021; Tilocca et al., 2020). Among these microorganisms, bacteria play a crucial role in the development of flavor and aroma in cheese (Chen et al., 2017). The bacterial communities in cheese can be divided into two main categories: starter cultures and non-starter bacteria (Gaglio et al., 2020). Starter cultures are a defined mixture of lactic acid bacteria that are added to milk at the beginning of cheese production to initiate the fermentation process (Gaglio et al., 2020). The most common lactic acid bacteria used as starter cultures include *Lactococcus lactis*,

Streptococcus thermophilus, and *Lactobacillus helveticus* (Zheng et al., 2021). These bacteria ferment lactose in milk to produce lactic acid, which lowers the pH of the milk and causes it to coagulate into curd (Chen et al., 2017). The non-starter bacteria, on the other hand, are bacteria that are naturally present in milk or added to cheese during the production process (Gaglio et al., 2020). These bacteria can contribute to the flavor and aroma of cheese by producing a range of volatile compounds, such as diacetyl acetaldehyde, and 2-butanone (Zheng et al., 2021). Non-starter bacteria can also contribute to cheese spoilage and quality issues, and their presence and activity need to be monitored closely during cheese production (Chen et al., 2017).

The consumption of cheese with bacterial flavor is associated with potential health benefits and safety concerns. Some studies have suggested that the presence



of lactic acid bacteria in cheese can provide health benefits, such as improved gut health and immune function. For example, one study found that the consumption of cheese containing probiotic strains of *Lactobacillus* can improve gut microbiota and reduce inflammation in the body (Kim et al., 2018). However, the presence of certain bacteria in cheese can also pose safety concerns.

Pathogenic bacteria such as *Listeria monocytogenes* and *Staphylococcus aureus* can be found in cheese and can cause foodborne illness (Button & Dutton, 2012). The presence of these bacteria in cheese can be a result of contamination during the production process, inadequate processing, or improper storage conditions (Hoel et al., 2019). It is, thus, essential to understand the potential health benefits and safety concerns associated with different types of cheese and to make informed choices based on individual needs and preferences. The objective of the study is to investigate the role of microorganisms, specifically total bacteria, Lactic Acid Bacteria (LAB), and fungi, in the production of different cheese varieties. We aimed to evaluate the potential hazard to human health based on standard colony forming unit (CFU/ml) measurements obtained from various literature sources.

Materials & Method

To isolate the bacteria, the samples were serially diluted using sterile maximum recovery diluents (pH 7), 0.1 ml of the For LAB isolation, dilution was applied to de Man, Rogosa, and Sharpe (MRS) agar. Agar plates were incubated for 24–48 hours at 37°C. (Possas et al., 2021) Potato Dextrose Agar (PDA) is made by boiling two hundred grams of peeled and sliced potatoes in one liter of distilled water for thirty minutes to make potato infusion. Pass the potato infusion-containing filtrate through cheesecloth, preserving the effluent (or use previously purchased dehydrated form). Boil to dissolve after mixing with dextrose, agar, and water. Autoclave at 121°C for 15 minutes. Divide the contents of 20–25 milliliters into sterile 15 x 100 mm petri dishes. Final pH, 5.6 ± 0.2 . (Aryal, 2022)

To investigate the microbial diversity in market cheese varieties, three different media were used: Nutrient agar for total bacteria count (Aryal, Nutrient Agar- Principle, Composition, Preparation, Results, Uses, 2022), MRS (de Man, Rogosa, and Sharpe) agar for LAB count, and PDA (Potato Dextrose Agar) for fungi count. The samples of soft cheese, full-fat sour cheese, sour cheese, traditional Chinese cheese, and blueberry cheese were

collected and analyzed using these media. The colonies were counted and recorded to determine the microbial composition of each cheese variety.

Statistical Analysis

To probe the intricate interplay between microorganisms and cheese, we employed a statistical symphony. Firstly, we harnessed the power of descriptive statistics and ANOVA to unveil the variations in colony-forming units (CFUs) for three key players: total bacteria, Lactic Acid Bacteria (LAB), and fungi. This analysis meticulously assessed the differences in microbial populations across five distinct cheese types, like a conductor illuminating the unique melody of each cheese.

To assess the potential health hazards lurking within these variations, we applied Principal Component Analysis (PCA) and machine learning statistics. PCA elegantly choreographed the complex relationships between CFUs and cheese types, revealing latent patterns in the microbial tapestry. With these patterns in hand, machine learning algorithms meticulously compared the CFU data against established microbiological quality standards for cheese, meticulously evaluating the likelihood of hazard for each cheese variety.

Microbiological Quality Standards for Cheese:

- **Total Plate Count Limit:** International standards set a limit of 105 CFU/mL for the total plate count in cheese, ensuring an assessment of overall microbial load within acceptable limits. (International Microbiological Criteria for Dairy Products, 2003);
- **Specific Pathogens:** Soft cheese should be free from *Listeria monocytogenes* (absence in 25 grams) and *Salmonella* (absence in 1 gram) according to the European Commission's criteria, safeguarding against known health risks. (International Microbiological Criteria for Dairy Products, 2003);
- **Lactic Acid Bacteria (LAB):** LAB, beneficial bacteria in cheese, should fall within the range of 104cfu/mL minimum range in 2.1 & maximum range is 3.5. (Guetouache & Guessas, 2015);
- **Fungi Count:** Acceptable fungi standard for cheese is between 1.2×10^2 cfu/ml. (Guetouache & Guessas, 2015).

Results

We found maximum variation in colony forming unit in case of traditional Chinese cheese, followed by soft cheese, sour cheese, blue berry cheese and full fat sour

cheese (Figure 1-A). Moreover, the colony forming unit was also found in variation for three target microorganisms (Figure 1-B). Among these microorganisms, total bacteria formed maximum colony in the traditional Chinese cheese. Furthermore, traditional Chinese cheese attained highly

discriminating features among the experimental cheeses (Figure 1-C). It is, thus, expected from both the ANOVA and Linear Discriminate analysis that colony forming unit of total bacteria and fungi is discriminatingly higher in traditional Chinese cheese, followed by soft cheese (Figure 1).

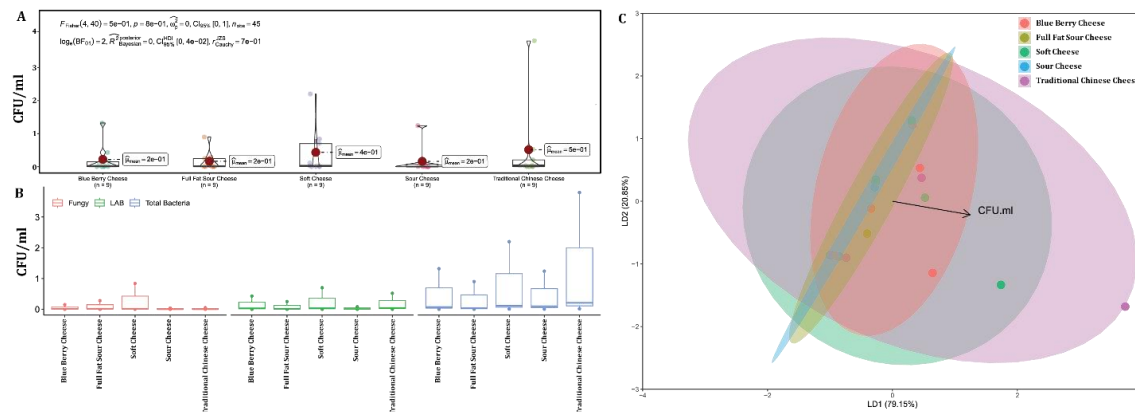


Fig-01: Microbial variability among the experimental cheeses. A: Analysis of Variance (ANOVA) of colony forming unit (CFU/ml) on different cheeses; B: Mean variation of the CFU/ml of fungi, LAB and TB on different cheeses; C: Linear Discriminate analysis of the CFU/ml on the cheeses.

We also produced heatmap to analyze the relative count among the three colony forming microorganisms in the five types of cheeses (Figure 2). We, here, found that the fungi count was upregulated in the traditional Chinese cheese, soft cheese and sour cheese under the PDA medium relative to the lactic acid bacteria and total bacteria (Figure 2-A & -B). In contrast, lactic acid bacteria and total bacteria was upregulated in the full fat sour cheese under the MRS medium relative to the fungi. Moreover, lactic acid bacteria was upregulated in the full fat sour cheese in compare to the total bacteria (Figure 2-C). Therefore, it is assumed that the full fat sour cheese is more subjected to more growth of lactic acid bacteria.

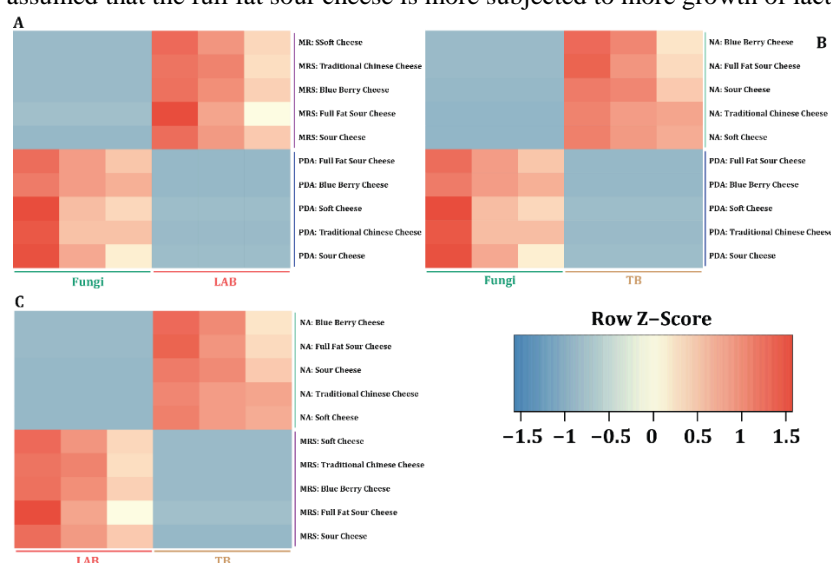


Fig-02: Heatmap of microbial count in different cheese under different medium. A: Relative composition of fungi and LAB; B: Relative composition of fungi and TB; C: Relative composition of LAB and TB



We calculated the likelihood of hazard risk for the experimental cheeses caused due to colony forming unit per ml of the three microorganisms based on the colony forming standard for the cheese. We found that no cheese exited the standard limit. However, these cheeses were likely to have chance to be health hazard to human. We found that total bacteria has high chance to cause all the cheeses, particularly the soft cheese, health hazard (**Figure 3-A**). However, the hazard likelihood was highly increased with increasing colony forming unit of fungi, which was highly expected to cause soft cheese health hazard (**Figure 3-B**). We also applied the PCA to analyze the hazard sensitivity of the five types of cheeses

in response to the three microorganisms (**Figure 3-C & -D**). We also found that traditional Chinese cheese become sensitive to health hazard by 201-300, soft cheese by 100-300, and blue berry cheese by 100-200 colony count of microorganism.

Finally, using machine learning discriminating method (LDA), we also calculated the posterior probabilities of the health hazard to assume the probable hazardous impact of the cheeses available in the Chinese market in response to the microbial growth (**Figure 4**). We found that the total bacteria has high probabilities to be health hazard with consuming traditional Chinese cheese from the market.

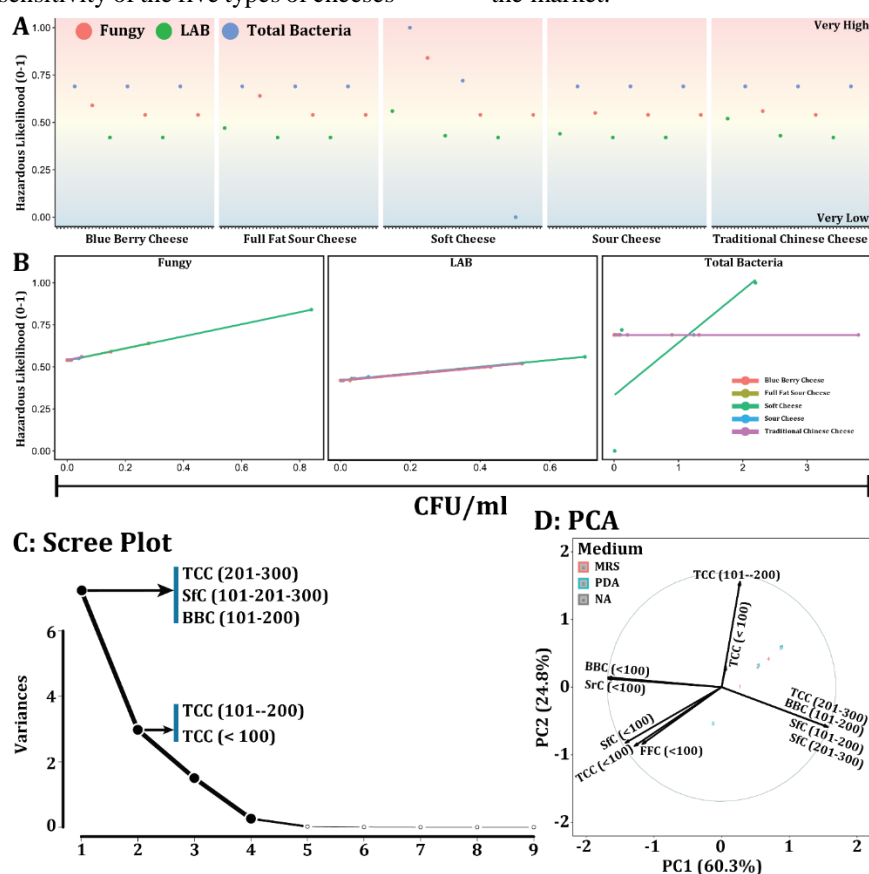


Fig-03: Hazard sensitivity analysis for different cheeses. A-B: Hazard likelihood of different cheeses in response to microbial exposure; C-D: Principal Component Analysis (PCA) for identify principal hazardous condition for cheeses; TCC: Traditional Chinese Cheese; BBC: Blue Berry Cheese; SFC: Soft Cheese; SrC: Sour Cheese; FFC: Full Fat Sour Cheese

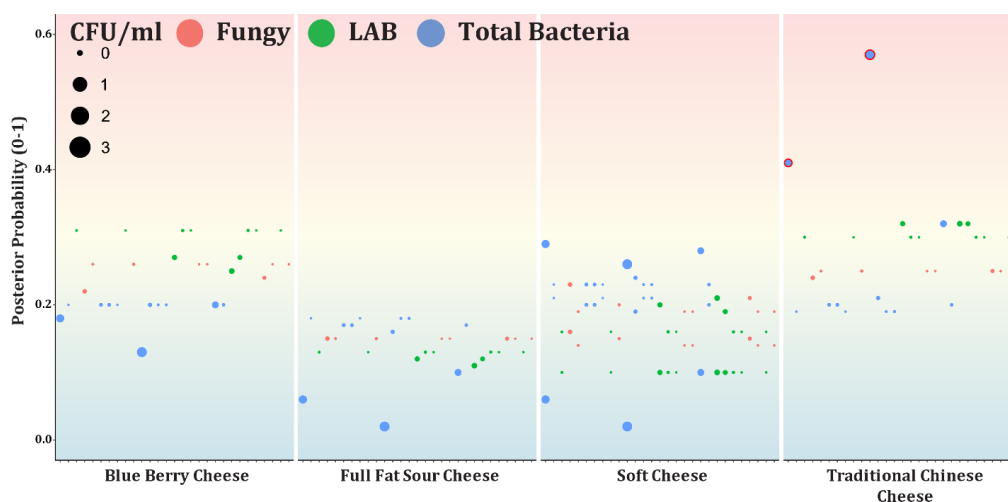


Fig-04: Posterior probability of hazardous impact of microbial growth in different cheeses

Discussion

The present study revealed that all the subjective cheeses are significantly exposed to total bacteria-driven contamination followed by Fungi. It is reported that producers should keep counts at $<5,000$ CFU/ml and counts of $>10,000$ CFU/ml are usually indicative of a problem (D'amico, 2014). Moreover, coliform counts should be <10 CFU/ml, although levels of <100 CFU/ml are often considered acceptable for the manufacture of cheese (D'amico, 2014). Counts between 100 and 1,000 CFU/ml often indicate poor milking hygiene, while counts exceeding 1,000 indicate that bacterial multiplication is occurring somewhere along the line. (D'amico, 2014). It is, thus, expected that all types of microorganisms, particularly total bacteria and fungi, significantly increase and are about to exit the quality standards for Traditional Chinese Cheese and Soft Cheese.

Nevertheless, our study found lower colony-forming units in the case of lactic acid bacteria in all types of cheeses. It has been reported that during the long freezing storage of cheese, the reduction in lactic acid bacteria is typically a result of the low temperatures inhibiting the growth and activity of these bacteria (Button & Dutton, 2012). This finding indicates that storage facilities might restrict the growth of lactic acid bacteria, and consequently their colony-forming capacity.

Traditional Chinese cheese and soft cheese may undergo distinct cheese-making processes. The methods employed could create conditions favoring the proliferation of specific bacterial strains, leading to

higher CFU/ml counts. (Possas et al., 2021) Differences in ingredients, such as milk types, cultures, and any unique additives, can contribute to varying microbial populations. Traditional Chinese cheese and soft cheese may have specific components influencing bacterial growth (Possas et al., 2021). Moreover, the duration and conditions of fermentation can significantly impact bacterial counts (Possas et al., 2021). Our study expected that both Traditional Chinese Cheese and Soft Cheese might undergo a fermentation process that promotes the growth of certain bacteria, like total bacteria and fungi. Furthermore, the texture and moisture content of cheeses play a role in specific bacterial growth (Possas et al., 2021). Soft cheese, with its distinct texture and higher moisture, may support different microbial communities compared to Traditional Chinese cheese. (Possas et al., 2021). It is, thus, concluded that traditional Chinese cheese and soft cheese may be at high risk of causing health hazards to humans against total bacteria and fungi. One of the major limitations of the present study is not to evaluate the limiting factors underlying specific microbial growth. To fully comprehend the results, it's crucial to delve into the specific details of the cheese-making processes, ingredient compositions, and fermentation conditions for both Traditional Chinese cheese and soft cheese. Analyzing these factors will provide a comprehensive understanding of why these two types of cheese exhibited the maximum CFU/ml counts in the study.

Conclusion

Microorganisms, particularly bacteria and fungi, play a vital role in the production of cheese. The microbial



diversity in different cheese varieties contributes to their distinct flavors, textures, and characteristics. Understanding the role of microorganisms in cheese production can help in the development of new cheese varieties and improve the quality of existing ones. Further research in this field can lead to innovations in cheese production techniques and enhance the overall cheese-making process.

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