

MICROBIOLOGICAL ASSESSMENT OF SOY MILK COLLECTED FROM RAMADAN BAZAAR IN KUALA NERUS, TERENGGANU

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<https://doi.org/10.46754/umtjur.v7i1.395>

Received: 25 April 2023

Revised: 14 November 2024

Accepted: 14 November 2024

Published: 15 April 2025

Abstract: Soy milk is a popular non-dairy alternative, often consumed for its high protein content and health benefits. Despite its increasing popularity, concerns about the quality and safety of soy milk persist. Soy milk sold at the Ramadan bazaar is typically home-prepared by small-scale producers, making it susceptible to contamination due to its rich nutritional profile and simple packaging. This can lead to a short shelf life and potential public health issues. Hence, this study aimed to assess the microbial quality of soy milk samples from the Ramadan bazaar in Kuala Nerus. A total of 48 soy milk samples were randomly purchased from the Ramadan bazaar Gong Badak, Kuala Nerus, between 20th May and 10th June 2018. The Total Plate Count method was used to determine the bacterial load in the soy milk, while XLD agar and the BBL Crystal Kit were employed to identify isolated bacteria. The study reported that the total bacterial count ranged from $2.81 \pm 0.08 \log_{10}$ CFU/ml to $9.71 \pm 0.04 \log_{10}$ CFU/ml, with the highest count exceeding the allowable limit for soy beverages ($5 \log_{10}$ CFU/ml). Additionally, 62.5% of the selected screened samples revealed coliform bacteria, the *Cronobacter sakazakii* complex, *Acinetobacter baumannii*, *Enterobacter cloacae*, *Salmonella* sp., and *Escherichia coli*. These findings highlight the significance of proper management during soy milk production to prevent contamination that could lead to foodborne illnesses. Regular screening and monitoring of food and beverages sold at markets and Ramadan bazaar are essential to enforce food safety and security measures.

Keywords: Food safety, foodborne disease, foodborne pathogens, food sampling.

Introduction

Soy milk has emerged as a popular non-dairy alternative, recognised for its high protein content and various health benefits. It serves as an excellent source of plant-based protein, making it particularly advantageous for individuals who are lactose intolerant, vegan, or aiming to limit animal product consumption. Remarkably, a typical serving of soy milk contains approximately 7 g of protein, which is comparable to cow's milk.

Beyond its protein profile, soy milk contains essential nutrients including vitamins, minerals, and antioxidants. These components contribute to its status as a health-promoting beverage. Notably, isoflavones in soy milk are known for their antioxidant properties and potential to reduce inflammation, enhancing its health benefits. Additionally, soy milk is associated with cardiovascular health due to its omega-3

fatty acids and potassium content which can help lower blood pressure and cholesterol levels (Saini & Morya, 2021). Furthermore, the cost-effectiveness and widespread availability of soy milk further contribute to its growing consumption. Soybeans, the primary ingredient in soy milk, are relatively inexpensive to cultivate, making soy milk a budget-friendly option for consumers. Moreover, soy milk can be easily found in supermarkets, health food stores, and local markets, including the Ramadan bazaar which is known for offering various home-prepared food and beverages.

Despite its benefits, the quality and safety of soy milk, particularly those sold at local bazaars such as Ramadan bazaar, remain significant concerns. These products are often produced by small-scale manufacturers using home-based methods which can compromise

safety standards. The rich nutritional profile of soy milk, while advantageous, also makes it highly susceptible to microbial contamination. This risk is compounded by inadequate packaging practices leading to a shorter shelf life and potential health hazards for consumers. Research highlights that the presence of harmful compounds such as allergens and anti-nutritional factors, can pose health risks associated with soy milk consumption. In particular, allergens observed in soybeans can trigger reactions ranging from mild to severe in sensitive individuals. Furthermore, biogenic amines which may form during fermentation, can have adverse health effects if not properly managed during production (Messina, 2016). Effective processing methods including soaking, grinding, and heating, are critical in mitigating these harmful compounds. Nevertheless, they may not always be utilised in small-scale production settings (Saini & Morya, 2021).

The risk of microbial growth in soy milk is heightened due to its near-neutral pH and high moisture content, making it essential for producers to implement stringent safety measures to ensure product quality and consumer safety (Messina, 2016). Additionally, studies have suggested that soy milk can harbour pathogenic bacteria if proper hygiene practices are not followed during preparation. This further underscores the need for improved safety protocols in small-scale production (Messina, 2016; Saini & Morya, 2021).

Numerous studies have highlighted the nutritional advantages of soy milk. For instance, Messina (2016) emphasised that soy milk is a valuable source of isoflavones linked to various health benefits, including reduced risks of heart disease and certain cancers (Jooyandeh, 2011). Additionally, soy milk provides a balanced profile of essential amino acids, making it an ideal protein source for those who do not consume animal products. Specifically, its low saturated fat content and absence of cholesterol further enhance its reputation as a heart-healthy beverage. Research indicates that the consumption of soy milk can contribute

to cardiovascular health by helping to lower cholesterol levels and improve overall heart function (Messina, 2016; Saini & Morya, 2021). Moreover, isoflavones in soy milk are associated with anti-inflammatory properties, which may further support its role in chronic disease prevention (Messina, 2016).

However, the safety of soy milk, particularly in small-scale production settings, remains a topic of concern. Studies by Zhang *et al.* (2008) have revealed that improper management and storage conditions can lead to significant microbial contamination. The nutritious nature of soy milk, combined with inadequate food processing and storage, creates a suitable environment for the growth of spoilage organisms (Stanley *et al.*, 2014). In addition, common pathogens identified in contaminated soy milk include *Escherichia coli*, *Salmonella* sp., and various coliform bacteria, which can cause foodborne illnesses. The simplicity of soy milk's packaging and the lack of pasteurisation in many small-scale productions exacerbate these risks (Kwok *et al.*, 2002). Moreover, research has indicated that the near-neutral pH and high moisture content of soy milk contribute to its susceptibility to microbial growth, necessitating stringent safety measures during production (Messina, 2016). In essence, the presence of allergens and anti-nutritional factors in soy milk can pose additional health risks if not properly managed (Saini & Morya, 2021).

Research focusing on food safety practices in local markets, such as the Ramadan bazaar, is limited but critical. Previous investigations by Jahan *et al.* (2018) indicated that vendors often lack adequate knowledge of hygiene practices, contributing to contamination risk. The unpasteurised food and lack of preservatives could contaminate food with various microorganisms such as fungi, coliform, and aerobic mesophilic bacteria (Deepika & Shahi, 2017). Correspondingly, ingesting contaminated food could lead to infection in the alimentary tract due to toxins released by the microorganisms, thus damaging the epithelium and causing gastroenteritis (Mohammad *et*

al., 2018). Considering this perspective, this study builds on existing literature by providing a detailed microbial analysis of soy milk sold at the Ramadan bazaar, highlighting the need for stringent food safety measures and regular monitoring. Note that by ensuring that vendors adhere to proper handling and hygiene protocols, the safety and quality of soy milk can be significantly improved, thereby protecting public health.

Materials and Methods

Sampling and Sample Processing

The 48 soy milk samples were purchased from Ramadan bazaar in Kuala Nerus from 20th May to 10th June 2018. The soy milk samples were aseptically managed and stored, without opening the package, at -21°C in the laboratory refrigerator until further analysis.

Total Viable Count

The sample was prepared by diluting 1 ml of soy milk with 9 ml of 0.85% saline solution. Each sample was diluted into a 5-fold dilution and spread onto a nutrient agar plate. Nutrient agar (HiMedia, India) was prepared according to the manufacturer's instructions. The colonies were counted after 24 hours of incubation at 37°C using a colony counter, and the total number of bacteria was calculated using the following formula:

Total number of bacteria in Colony Forming Unit (CFU) = (number of colonies)/[(dilution) × (actual volume plated)].

Detection and Identification of Bacteria

The soy milk sample was cultured on XLD agar before incubation for 24 hours at 37°C. The coliform bacteria appear yellow in colour on XLD agar while *Salmonella* appears red and *Salmonella typhi* form red colonies with black centers. Consequently, the preceding yellow and red colonies on XLD agar were identified using the BBL Crystal kit (Becton Dickinson, USA). The selected bacteria colony was first

suspended in the BBL Crystal Inoculum Fluid to a turbidity equivalent to 0.5 McFarland Standard. The samples were transferred into the target area of the base and subsequently incubated for 24 hours. Following incubation, distinct chromogenic and fluorogenic reactions generate a profile number that is keyed directly into the BBL Crystal MIND Software installed on a laboratory computer.

Results and Discussions

Bacterial Burden in the Soy milk

Colony Formation Unit (CFU) per milliliter is used to reflect the number of viable bacteria present in the soy milk samples and determine their safety for consumption. The mean value of the Total Viable Count (TVC) of soy milk is presented in Figure 1. Generally, the mean bacterial count across most premises over the four sampling weeks was higher than the acceptable limit of 5 log₁₀ CFU/ml by the Malaysian Regulatory Standards in the Fifteenth Schedule: Regulation 39 of the Malaysia Food Act 1983 and Food Regulation 1985. The TVC of bacteria in soy milk is a critical indicator of its safety and quality. Note that high TVC can result from various factors including contamination during production, improper handling, inadequate storage conditions, and using contaminated raw materials (Mollakhalili-Meybodi *et al.*, 2022).

In contrast, a low TVC typically indicates effective sterilisation processes, adherence to Good Manufacturing Practices (GMP), and proper storage. To maintain a low TVC, it is essential to implement rigorous quality control measures. In particular, regular microbiological testing, stringent sanitation protocols, and comprehensive employee training are vital to ensuring product safety. Additionally, post-contamination during packaging is a significant concern. Simultaneously, ensuring a clean and sanitised packaging environment, using aseptic packaging techniques, and handling the product correctly can minimise this risk.

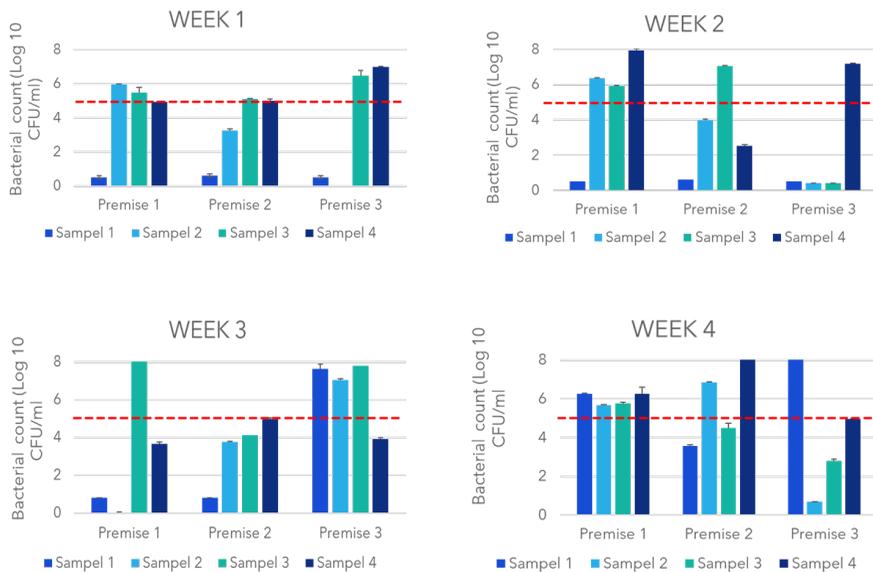


Figure 1: Total Plate Count of soy milk from three premises from week 1 to week 4. Data are presented as mean \pm standard deviation ($n = 3$) (\log_{10} CFU/ml). The limit of quantification is $1 \log_{10}$ CFU/ml. The limit of acceptable amount (dotted line) is $5 \log_{10}$ CFU/ml by the Malaysian Regulatory Standards in the Fifteenth Schedule: Regulation 39 of the Malaysia Food Act 1983 and Food Regulation 1985

A total of 48 soy milk samples collected from three different Ramadan bazaar premises were cultured on XLD selective media. After 24 hours of incubation, colonies with different colours were observed. Based on the results displayed in Figure 2, coliforms and *Shigella* bacteria were present each week. Conversely, *Salmonella* was detected only during week 3, comprising 4% of the samples. It was noted that the total amount of coliform bacteria was higher than *Shigella* and *Salmonella* each week.

To better understand these findings, further discussion and recent comparative studies on bacterial isolation techniques in soy milk should be considered. Traditional methods like direct plating, are commonly used for isolating bacteria such as *Listeria monocytogenes*.

However, molecular techniques, including Polymerase Chain Reaction (PCR) and Quantitative PCR (qPCR), have demonstrated higher sensitivity and specificity. For example, Most Probable Number (MPN) techniques combined with PCR can complete detection in two days and provide higher accuracy by targeting specific DNA sequences of the bacteria. This overcomes the limitations of traditional methods like labour intensiveness and interference from background microflora (Mollakhalili-Meybodi *et al.*, 2022). Such advancements highlight the potential for improved detection and quantification of bacterial contamination in soy milk, ensuring better quality control and reducing the risk of post-packaging contamination.

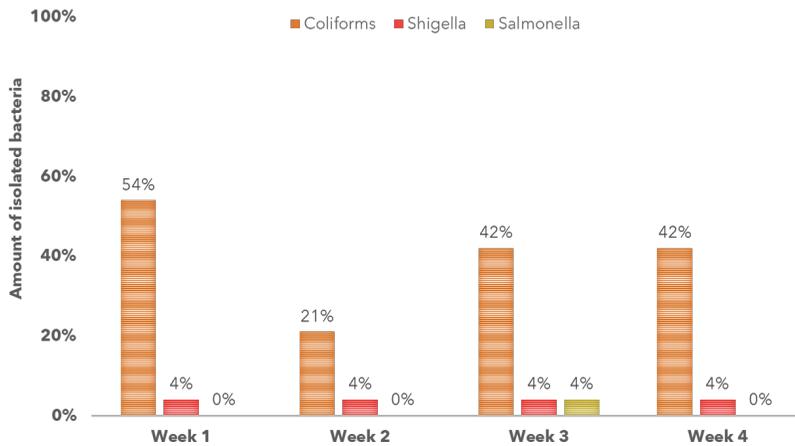


Figure 2: Bacterial occurrence on selective XLD agar from 48 soy milk samples collected in three Ramadan bazaar premises

Bacterial identification by BBL Crystal

The colonies grown on the XLD agar were subsequently selected for further identification using the BBL Crystal kit. Due to constraints such as the limited availability of testing resources and the need for efficient use of laboratory time and materials, only four representative bacterial colonies were subjected to BBL Crystal identification. The results revealed the bacterial species present on the XLD agar, as detailed in Table 1. This selective approach ensured that the most representative samples were analysed while maintaining the reliability of the identification process.

Cronobacter sakazakii is predominantly found in dairy products, vegetables, and spices (Hunter *et al.*, 2008). This microbe is notorious for its lethality, especially in cases involving infant infections (Lepuschitz *et al.*, 2019).

Additionally, *Acinetobacter baumannii* was also isolated from the soy milk sample. This Gram-negative bacterium is a major cause of nosocomial infections. It is classified as one of the ESKAPE pathogens (*Enterococcus* sp., *Staphylococcus aureus*, *Klebsiella pneumoniae*, *A. baumannii*, *Pseudomonas aeruginosa*, and *Enterobacter* spp.) due to its virulence and antibiotic resistance (Morris *et al.*, 2019). Notably, *Acinetobacter* can infect human skin, wounds, and the respiratory and gastrointestinal tracts. It is known for surviving in harsh environments for weeks, enhancing its virulence (Dahiru & Enabulele, 2015). Limited data on *A. baumannii* isolates from food products such as meat, vegetables, and milk suggests a potential interchange of bacteria between environments and food (Askari *et al.*, 2020).

Table 1: Bacterial colony morphology description and identification by BBL Crystal of four representative isolated bacteria grown on XLD agar

Colony Morphology on XLD Agar	Species Name	ID Confidence Level
Medium-sized, circular, glistening, yellow	<i>Cronobacter sakazakii</i> complex	0.9993
Medium-sized, domed, mucoid, yellow	<i>Acinetobacter baumannii</i>	0.9851
Large-sized, smooth, flat, entire, yellow	<i>Enterobacter cloacae</i>	0.9873
Medium-sized, smooth, flat, red with a black dot	<i>Salmonella</i> sp.	0.8992

Another species reported in the soy milk sample is *Enterobacter cloacae*, which is typically found in water and soils and is frequently isolated from humans and animals (Cao et al., 2020). This species is known for its resistance to the carbapenem class of antibiotics and can cause infections in humans, leading to pneumonia, septicemia, and urinary tract infections (Annavajhala et al., 2019).

In addition, a red colony was observed on XLD agar which was identified as *Salmonella* sp. These bacteria are widespread pathogens capable of causing zoonotic infections in animals and humans (LeLièvre et al., 2020). *Salmonella enterica*, a prominent species within this genus is a leading cause of global foodborne illness, resulting in diarrheal and gastroenteritis symptoms. Globally, *Salmonella* infections are responsible for approximately 94 million cases and 155,000 deaths annually (Vinayaka et al., 2019). Lastly, a yellow colony identified as *E. coli* was also observed on XLD agar. *E. coli*, commonly found in vegetables, water, uncooked food, meat, and unpasteurised milk, is notorious for causing severe illnesses such as kidney failure and hemorrhagic gastroenteritis (Li et al., 2020).

Conclusions

Data from this study revealed that the microbial content in the soy milk samples significantly exceeded the Malaysian regulatory standard of $5 \log_{10}$ CFU/ml, with all samples exhibiting a high prevalence of coliform bacteria. The isolation and identification of specific bacterial strains might also suggest that cross-contamination likely occurred during the soy milk production process. These findings underscore the need for enhanced hygiene practices to produce non-commercial soy milk. To mitigate bacterial contamination in soy milk production, it is essential to implement several precautionary measures. These include regular microbiological testing to monitor bacterial levels, strict adherence to personal hygiene protocols among producers, and establishing proper storage systems to prevent the growth of harmful microorganisms.

This study highlights the critical need for non-commercial soy milk producers and sellers to adopt these preventive measures to reduce the risk of foodborne illnesses among consumers. Moreover, comprehensive training on soy milk hygiene and quality control is vital. In addition, producers should be educated on the importance of quality testing, maintaining sanitary production environments, and using proper refrigeration systems to ensure that soy milk remains safe for consumption. Nevertheless, by implementing these strategies, non-commercial soy milk producers can significantly improve the quality and safety of their products, protecting public health.

Acknowledgments

The authors would like to acknowledge the Biological Sciences laboratory at the Faculty of Science and Marine Environment, Universiti Malaysia Terengganu, for assistance and support in this study.

Conflict of Interest Statement

The authors declare that they have no conflict of interest.

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