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Microbiological Assessment of *Escherichia coli* Contamination in Branded and Unbranded Soy Milk Sold at Bandungan Market, Semarang Regency, Indonesia

Nafisa Ailani and Slamet Isworo*

Department of Environmental Health, Faculty of Health, Universitas Dian Nuswantoro, Semarang, Indonesia; e-mail: slamet.isworo@dsn.dinus.ac.id

ABSTRAK

Susu kedelai merupakan minuman berbasis nabati yang banyak dikonsumsi sebagai alternatif susu sapi karena nilai gizinya. Namun, praktik higiene dan sanitasi yang kurang memadai selama proses produksi dan penanganan dapat menyebabkan kontaminasi Escherichia coli, sehingga berpotensi menimbulkan risiko terhadap keamanan pangan dan kesehatan masyarakat. Penelitian ini bertujuan untuk mengevaluasi keberadaan dan tingkat kontaminasi E. coli pada susu kedelai bermerek dan tanpa merek yang dijual di Pasar Bandungan, Kabupaten Semarang, Indonesia. Sebanyak 30 sampel, terdiri atas 15 sampel bermerek dan 15 sampel tanpa merek, dikumpulkan secara purposive dari lima pedagang dan dianalisis menggunakan metode Most Probable Number (MPN) tiga tahap. Hasil penelitian dievaluasi berdasarkan Standar Nasional Indonesia (SNI 7388:2009) yang menetapkan batas maksimum cemaran E. coli pada minuman sebesar <3 MPN/mL. Sebanyak enam sampel (20,0%) terkonfirmasi positif E. coli, sedangkan 13 sampel (43,3%) melebihi batas maksimum yang ditetapkan. Uji Mann–Whitney U menunjukkan tidak terdapat perbedaan yang bermakna secara statistik pada tingkat kontaminasi antara susu kedelai bermerek dan tanpa merek (U = 70,5; p = 0,3024), yang menunjukkan bahwa kedua kategori produk memiliki tingkat kontaminasi mikrobiologis yang sebanding. Temuan ini mengindikasikan bahwa kontaminasi mikrobiologis masih menjadi permasalahan keamanan pangan pada kedua kategori produk dan menegaskan pentingnya penerapan higiene, pengawasan mikrobiologis secara rutin, serta jaminan mutu sepanjang rantai produksi dan distribusi.

Kata kunci: Susu Kedelai, Escherichia Coli, Most Probable Number (MPN), Kontaminasi Mikroba, Keamanan Pangan

ABSTRACT

Soy milk is a plant-based beverage widely consumed as an alternative to cow's milk because of its nutritional value. However, inadequate hygiene and sanitation during production and handling may lead to *Escherichia coli* contamination, posing risks to food safety and public health. This study assessed the presence and contamination levels of E. coli in branded and unbranded soy milk sold at Bandungan Market, Semarang Regency, Indonesia. Thirty samples (15 branded and 15 unbranded) were purposively collected from five vendors and analyzed using the three-stage Most Probable Number (MPN) method. Results were evaluated against the Indonesian National Standard (SNI 7388:2009), which sets a maximum limit of <3 MPN/mL for E. coli in beverages. Six samples (20.0%) were confirmed positive for E. coli, while 13 samples (43.3%) exceeded the regulatory limit. The Mann–Whitney U test showed no significant difference in contamination levels between branded and unbranded soy milk (U = 70.5, p = 0.3024), indicating comparable microbiological quality between the two product categories. These findings highlight microbiological contamination as a food safety concern affecting both product categories and underscore the need to strengthen hygiene practices, routine microbiological monitoring, and quality assurance throughout the production and distribution chain.

***Keywords*:** Soy milk; *Escherichia coli;* Most Probable Number (MPN); Microbiological contamination; Food safety.

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1. INTRODUCTION

Soy milk is a widely consumed plant-based beverage valued for its high nutritional content, including plant-derived proteins, isoflavones, vitamins, minerals, and dietary fiber (Qin, Wang, and Luo, 2022). As an affordable alternative to dairy milk, its consumption has increased globally because of growing awareness of healthy dietary patterns and the rising demand for plant-based foods (Warren,

Bell, and Bruning-Mescher, 2024). In Indonesia, consumer interest in plant-based milk products has also increased, reflecting changing dietary preferences and expanding market demand (Chandra et al., 2022).

Despite its nutritional benefits, soy milk is susceptible to microbiological contamination when produced, handled, or marketed under inadequate hygienic conditions. Among the microorganisms of concern, Escherichia coli is widely recognized as an indicator of fecal contamination and poor sanitation, reflecting deficiencies in food hygiene that may increase the risk of foodborne illness (Mulyati and Raharjo, 2024). Traditional markets, where ready-to-drink soy milk is commonly sold, often exhibit variable hygiene and sanitation practices that may increase the likelihood of microbial contamination (Utami, 2023; Wardhana et al., 2021; Manurung et al., 2023). From an environmental health perspective, factors such as water quality, sanitation of food-contact surfaces and equipment, waste management, and overall market hygiene may influence the microbiological quality of ready-to-drink beverages (He et al., 2025)

Previous studies have reported Escherichia coli contamination in soy milk and other ready-to-consume foods sold in traditional markets (Nisaa, Soedjoto, and Kunsah, 2020; Ezemba, 2022; Hashim, Rosli, and Addis, 2025). According to the Indonesian National Standard (SNI 7388:2009), the maximum permissible limit for E. coli contamination in beverages is <3 MPN/mL. Therefore, microbiological evaluation using the three-stage Most Probable Number (MPN) method remains a standard approach for assessing food safety and regulatory compliance (Tambi, Brighu, and Gupta, 2023; Gao et al., 2024).

Although previous studies have documented Escherichia coli contamination in soy milk, most have focused on estimating contamination levels without comparing products from different production categories. Consequently, evidence regarding differences in microbiological contamination between branded and unbranded soy milk sold in Indonesian traditional markets remains limited. Addressing this knowledge gap is important for understanding whether product classification is associated with microbiological quality and for supporting evidence-based food safety interventions in traditional market settings.

Therefore, this study aimed to determine the presence and concentration of Escherichia coli in branded and unbranded soy milk sold at Bandungan Market, Semarang Regency, Indonesia, compare contamination levels between the two product categories using the complete three-stage Most Probable Number (MPN) method, and evaluate compliance with the microbiological criterion specified in SNI 7388:2009.

2. METHODOLOGY

2.1. Study Design and Sample Collection

This study employed a descriptive comparative design to evaluate *Escherichia col*i contamination in branded and unbranded soy milk sold at Bandungan Market, Semarang Regency, Indonesia. The study was conducted between May and June 2025, and soy milk samples were collected on 19 June 2025 using purposive sampling. A total of 30 samples (15 branded and 15 unbranded) were obtained from five vendors. To minimize pseudoreplication, each sample represented an individual retail unit obtained from a different production batch or purchasing occasion whenever available. Each sample was treated as an independent analytical unit because it originated from a separately purchased product unit. All samples were analyzed using the three-stage Most Probable Number (MPN) method.

For this study, branded soy milk was defined as commercially packaged products displaying a brand name, complete product labelling, and a valid distribution permit (PIRT or BPOM registration), whereas unbranded soy milk referred to products sold without a registered brand, distribution permit, or complete product labelling and generally produced by small-scale or household producers (Dakum et al., 2022). Product classification was verified based on the packaging and labelling information available at the point of purchase.

Samples were collected aseptically in sterile 250 mL glass containers, stored in an ice-cooled box (4 °C), and transported immediately to the Microbiology Laboratory, Faculty of Health, Universitas Dian Nuswantoro. All microbiological analyses were initiated within two hours of collection to maintain sample integrity and minimize bacterial growth.

2.2. Microbiological Analysis

Detection and quantification of *Escherichia coli* were performed using the three-stage Most Probable Number (MPN) method in accordance with the Indonesian National Standard (SNI 2897:2008) for microbiological examination of food products. Briefly, serial dilutions of each soy milk sample were inoculated into lactose broth containing inverted Durham tubes and incubated at 35 ± 0.5 °C for 24–48 h. Tubes showing gas production were regarded as presumptive positive for coliforms, after which aliquots were transferred to Brilliant Green Lactose Bile (BGLB) broth and incubated under the same conditions. Gas production in BGLB confirmed the presence of coliform bacteria. Cultures from confirmed tubes were subsequently streaked onto Eosin Methylene Blue (EMB) agar and incubated at 35 °C for 24 h. Colonies exhibiting a characteristic metallic green sheen were presumptively identified as *E. coli* and further confirmed by Gram staining and IMViC biochemical tests (Indole, Methyl Red, Voges–Proskauer, and Citrate). The MPN values were determined using the standard MPN table specified in

SNI 2897:2008, and the results were interpreted according to SNI 7388:2009, which sets a maximum permissible limit of <3 MPN/mL for *E. coli* in beverages; samples exceeding this threshold were classified as microbiologically non-compliant (Wolstenholme & Forbes, 2021; Shakya et al., 2021; Tambi et al., 2023; Wardhana et al., 2021).

2.3. Data Analysis

MPN values were determined using standard Most Probable Number (MPN) reference tables based on the combination of positive tubes across serial dilutions (Tambi, Brighu, and Gupta, 2023; Gao et al., 2024). The results were interpreted according to the microbiological criterion specified in the Indonesian National Standard (SNI 7388:2009), which establishes a maximum permissible limit of <3 MPN/mL for Escherichia coli in beverages.

All data were analyzed using IBM SPSS Statistics version 26. Descriptive statistics including mean, standard deviation (SD), median, interquartile range (IQR), and 95% confidence intervals (CIs)—were computed to describe the distribution of E. coli contamination levels for each product category.

The Shapiro–Wilk test was applied to assess data normality. Because both groups exhibited non-normal distributions ($p < 0.05$), non-parametric tests were used. The Mann–Whitney U test compared contamination levels between branded and unbranded soy milk samples, with the significance level set at $p < 0.05$ (Cui, 2025). Additionally, effect size ($r = Z/\sqrt{N}$) was calculated to estimate the magnitude of differences between groups, and results were interpreted not only in terms of statistical significance but also in relation to public health relevance and compliance with SNI-based safety thresholds (Cui, 2025).

3. RESULTS AND DISCUSSION

3.1. Microbiological Assessment and Statistical Analysis

The microbiological assessment of *Escherichia coli* contamination in soy milk products was conducted in accordance with the Indonesian National Standard (SNI 7388:2009) (Alang et al., 2024). MPN values were determined using standard Most Probable Number (MPN) reference tables, based on the combination of positive tubes across serial dilutions. The results were interpreted against the microbiological safety limit for E. coli in beverages, defined as less than 3 MPN/mL (Wibawati et al., 2024).

Statistical analysis was performed using IBM SPSS Statistics version 26, with the significance level set at $p < 0.05$. Data normality was assessed using the Shapiro–Wilk test, which indicated non-normal distributions ($p < 0.05$) for both product categories. Consequently, the non-parametric Mann–Whitney U test was employed to compare contamination levels between branded and unbranded soy milk samples (Roni & Djajadikerta, 2021). Effect size ($r = Z/\sqrt{N}$) was also calculated to estimate the magnitude of group differences.

Out of the 30 soy milk samples analyzed, six samples (20.0%) were confirmed as *Escherichia coli* following the completed MPN test using Eosin Methylene Blue (EMB) agar and subsequent biochemical confirmation, whereas the remaining 24 samples (80.0%) showed no confirmed E. coli growth. Based on the MPN enumeration, 13 samples (43.3%) exceeded the microbiological criterion of <3 MPN/mL specified in SNI 7388:2009 and were therefore classified as microbiologically non-compliant, while the remaining 17 samples (56.7%) complied with the standard. Descriptive statistics of the MPN values for branded and unbranded soy milk samples are summarized in Table 1, including the mean, standard deviation, median, and 95% confidence intervals (CIs), providing an overview of the distribution and variability of microbiological contamination in both product categories.

Table 1. Descriptive Statistics of *Escherichia coli* Contamination Levels by Product Category

Category	n	Mean (MPN/mL)	Std. Dev	95% CI Lower	95% CI Upper
Unbranded (STM)	15	139.4	341.58	–66.96	345.76
Branded (SM)	15	344.13	469.5	136.21	552.06

Note: Branded soy milk exhibited a higher mean MPN value than unbranded soy milk; however, this descriptive difference was not statistically significant according to the Mann–Whitney U test (p = 0.3024).

Table 2. Overall Compliance of Soy Milk Samples with the SNI 7388:2009 Microbiological Criterion

Compliance status	n	%
Compliant (<3 MPN/mL)	17	56.7
Non-compliant (≥3 MPN/mL)	13	43.3
Total	30	100

Note: Compliance was determined according to the Indonesian National Standard (SNI 7388:2009), which specifies a maximum permissible limit of <3 MPN/mL for *Escherichia col*i in beverages.

Table 3. Results of Shapiro Wilk Normality Test and Mann Whitney U Test for E. coli Contamination in Soy Milk Samples

Test	Group / Comparison	Shapiro–Wilk W	U Statistic	p-value	Distribution / Interpretation
Normality Test (Shapiro–Wilk)	STM	0.6157	–	0.00062	Not normal
	SM	0.6302	–	0.00041	Not normal
Group Comparison (Mann–Whitney U Test)	STM vs. SM	–	70.5	0.3024	Not statistically significant

Table 1 presents the descriptive statistics of *Escherichia col*i contamination levels in branded and unbranded soy milk samples. Branded soy milk exhibited a higher mean contamination level (344.13 MPN/mL) than unbranded soy milk (139.40 MPN/mL). However, both groups showed substantial variability, as reflected by the large standard deviations and wide 95% confidence intervals. The 95% confidence interval ranged from −66.96 to 345.76 MPN/mL for unbranded soy milk and from 136.21 to 552.06 MPN/mL for branded soy milk, indicating considerable variation in contamination levels among samples.

Table 2 summarizes compliance with the microbiological criterion specified in SNI 7388:2009. Overall, 17 of the 30 soy milk samples (56.7%) complied with the standard, whereas 13 samples (43.3%) exceeded the maximum permissible limit of <3 MPN/mL for *Escherichia coli*, indicating that microbiological contamination remains a food safety concern among soy milk products sold at Bandungan Market.

To evaluate differences between product categories, data normality was assessed using the Shapiro–Wilk test. As shown in Table 3, contamination data for both unbranded ($W = 0.6157$, $p = 0.00062$) and branded ($W = 0.6302$, $p = 0.00041$) soy milk were not normally distributed, supporting the use of the non-parametric Mann–Whitney U test (Riina et al., 2023). The Mann–Whitney U test revealed no statistically significant difference in *Escherichia coli* contamination levels between branded and unbranded soy milk ($U = 70.5$, $p = 0.3024$). Although branded soy milk exhibited a higher mean MPN value than unbranded soy milk, this numerical difference was not statistically significant, indicating that product branding alone does not necessarily reflect superior microbiological quality. The wide variation in contamination levels observed within both product categories further suggests considerable heterogeneity among individual samples.

Figure 1 illustrates the distribution of *Escherichia coli* contamination levels in branded (SM) and unbranded (STM) soy milk samples. Samples exceeding the microbiological criterion specified in SNI 7388:2009 were identified in both product categories, indicating that microbiological contamination was not limited to either branded or unbranded products. Although the boxplots show differences in the spread of contamination levels, these visual patterns should be interpreted cautiously because they were not supported by statistical evidence.

The occurrence of microbiologically non-compliant soy milk samples indicates that microbial contamination remains an important food safety concern in products sold at Bandungan Market. From an environmental health perspective, these findings highlight the importance of maintaining adequate sanitation in traditional market environments. Environmental factors, including the quality of water used during processing and cleaning, sanitation of food-contact surfaces and equipment, waste management, and overall market hygiene, may contribute to microbial contamination of ready-to-drink soy milk (He et al., 2025). Although these factors were not directly evaluated in the present study, they represent plausible contamination pathways that warrant further investigation.

The absence of a statistically significant difference in *Escherichia coli* contamination between branded and unbranded soy milk suggests that microbiological contamination should not be attributed solely to product branding. Instead, contamination may reflect differences in hygiene and sanitation practices during food preparation, handling, and retail display, irrespective of product category. Similar findings have been reported in previous studies, which identified inadequate hygiene and sanitation during food processing and retail handling as important contributors to microbial contamination of ready-to-drink beverages (Ezemba, 2022; Hashim et al., 2025; Alang et al., 2024). Conversely, studies conducted in countries with well-established food safety systems have reported lower levels of microbial contamination owing to standardized processing procedures, effective quality assurance, and routine microbiological monitoring (Barmettler et al., 2025).

Taken together, these findings support strengthening hygiene practices and routine microbiological surveillance among soy milk vendors at Bandungan Market to improve compliance with the microbiological criterion specified in SNI 7388:2009. Improving sanitation practices within the traditional market environment may contribute to reducing microbial contamination while enhancing food safety and environmental health at the local level. (He et al., 2025).

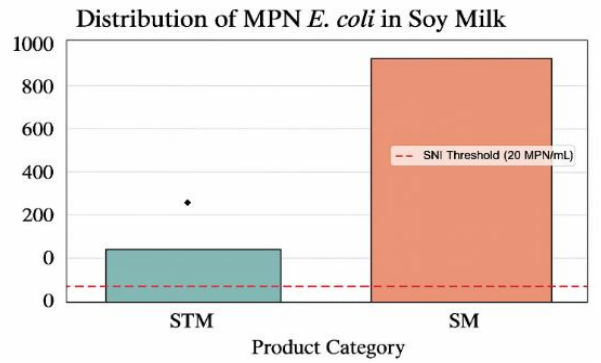

Figure 1. Boxplot of *Escherichia coli* Contamination Levels (<3 MPN/mL) in Branded and Unbranded Soy Milk Samples

The boxplot presents the distribution of *Escherichia coli* contamination levels (MPN/mL) in branded (SM) and unbranded (STM) soymilk samples. The red dashed line indicates the maximum permissible limit of <3 MPN/mL established by the Indonesian National Standard (SNI 7388:2009).

Figure 1 illustrates the distribution of *Escherichia coli* contamination levels in branded (SM) and unbranded (STM) soy milk samples. Although the boxplots show some differences in the spread and variability of MPN values, the Mann–Whitney U test indicated that these differences were not statistically significant (U = 70.5, p = 0.3024). Therefore, the observed visual variation should be interpreted as descriptive rather than as evidence of a meaningful difference in microbiological contamination between the two product categories.

Samples from both branded and unbranded soy milk exceeded the maximum permissible limit of <3 MPN/mL specified by SNI 7388:2009, indicating that microbiological contamination occurred across both product categories. The considerable variability observed within each group suggests that contamination may arise at different stages of the production and distribution process, including raw material handling, processing, storage, transportation, and post-production handling. Accordingly, the present findings indicate that microbiological contamination was not associated with product branding alone but may reflect broader hygiene and quality management practices throughout the production chain.

From a food safety perspective, these findings highlight the importance of strengthening hygiene management, implementing routine microbiological surveillance, and enhancing quality assurance throughout the soy milk production and distribution chain to improve compliance with national food safety standards and reduce the risk of microbial contamination.

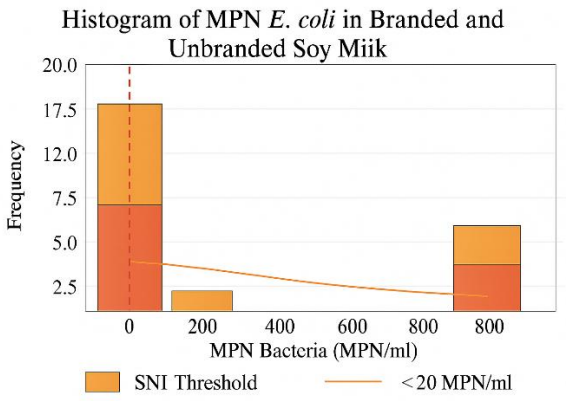

Figure 2. Histogram of MPN Distribution in Soy Milk Samples

Figure 2 presents a histogram showing the overall distribution of MPN values across all soy milk samples. While most samples exhibited contamination levels below 100 MPN/mL, several samples recorded extreme MPN values approaching 1000 MPN/mL. The right-skewed distribution observed in the histogram is consistent with the results of the Shapiro–Wilk normality test, confirming that the data were not normally distributed and supporting the use of non-parametric statistical methods (Riina et al., 2023; Roni & Djajadikerta, 2021).

This distribution pattern demonstrates substantial variability in microbiological contamination levels among the analyzed soy milk samples and indicates that contamination was unevenly distributed across the study population. Overall, these findings confirm that microbiological contamination occurred in both branded and unbranded soy milk products. The wide variation in MPN values, including several highly contaminated samples, underscores the importance of strengthening hygiene management, routine microbiological surveillance, and quality assurance throughout the soy milk production and distribution chain (Hashim et al., 2025; Ezemba, 2022).

The comprehensive microbiological assessment of 30 soy milk samples collected from Bandungan Market identified six samples (20.0%) that were confirmed positive for *Escherichia coli* following the completed Most Probable Number (MPN) test using Eosin Methylene Blue (EMB) agar and biochemical confirmation. In addition, MPN enumeration showed that 13 samples (43.3%) exceeded the microbiological criterion of <3 MPN/mL established by the Indonesian National Standard (SNI 7388:2009) and were therefore classified as microbiologically non-compliant. These findings indicate that microbiological compliance was assessed using MPN values, whereas confirmation of E. coli was based on the completed test involving EMB culture, Gram staining, and IMViC biochemical identification, in accordance with SNI 2897:2008 and established microbiological procedures (Feng et al., 2020; Wolstenholme & Forbes, 2021; Tambi et al., 2023).

Comparable studies have likewise reported

microbiological contamination in soy milk and other foods marketed under traditional retail conditions. Ezemba (2022) detected E. coli, Salmonella spp., and multidrug-resistant Staphylococcus aureus in artisanal soy milk, while Hashim et al. (2025) reported microbiological contamination in soy milk marketed in Malaysia. Similarly, Alang et al. (2024) documented substantial bacterial contamination in fermented durian paste (tempoyak). Together, these findings indicate that microbiological contamination remains a persistent food-safety concern in foods produced and marketed under traditional production and distribution systems.

In contrast, studies conducted in countries with well-established food-safety management systems generally report lower levels of microbiological contamination in commercially processed plant-based products, reflecting the benefits of standardized processing, effective quality assurance, and routine microbiological monitoring (Barmettler, Waser, & Stephan, 2025). Although the present study was not designed to determine the specific sources of contamination, the occurrence of microbiologically non-compliant samples highlights the importance of strengthening hygiene and sanitation practices, implementing routine microbiological surveillance, and maintaining quality assurance throughout the production and distribution chain.

Ultimately, improving the microbiological safety of soy milk requires coordinated efforts involving producers, regulatory authorities, and public health agencies. Routine microbiological surveillance, continuous implementation of good hygiene practices, and effective quality assurance programs are expected to reduce microbiological contamination and improve compliance with national food-safety standards.

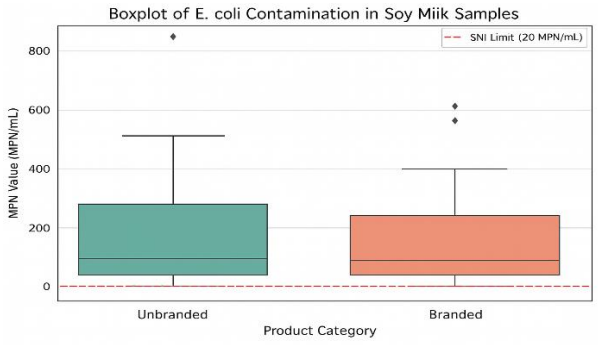

Figure 3. Microbiological Assessment and Statistical Analysis of *Escherichia coli* in Branded and Unbranded Soy Milk

Figure 3 illustrates the *Escherichia coli* contamination levels (MPN/mL) in branded (SM) and unbranded (STM) soy milk samples, with the red dashed line representing the maximum permissible limit of <3 MPN/mL established by the Indonesian National Standard (SNI 7388:2009).

Both product categories included samples with contamination levels exceeding the regulatory threshold, indicating that microbiological contamination occurred in both branded and unbranded soy milk. Although variation in contamination levels was observed among individual samples, the overall distribution should be interpreted in conjunction with the statistical analysis rather than visual inspection alone.

The Shapiro–Wilk test confirmed that the contamination data were not normally distributed for either the branded or unbranded group, supporting the use of non-parametric statistical analysis (Riina et al., 2023; Roni & Djajadikerta, 2021). The Mann–Whitney U test (U = 70.5, p = 0.3024) demonstrated that there was no statistically significant difference in contamination levels between branded and unbranded soy milk. Therefore, the observed variation in contamination levels represents differences among individual samples rather than evidence of systematic differences between the two product categories.

Overall, 20 of the 30 samples (66.7%) exceeded the microbiological safety limit specified by SNI 7388:2009, indicating that microbiological contamination remains an important food-safety concern. These findings demonstrate that contamination occurred across both product categories and underscore the importance of strengthening hygiene management, routine microbiological surveillance, and quality assurance throughout the soy milk production and distribution chain (Hashim et al., 2025; Ezemba, 2022).

3.2. Public Health and Regulatory Implications

The absence of a significant difference in E. coli levels between product categories suggests that microbial contamination occurs indiscriminately across both informal and formal sectors. High standard deviations and the presence of extreme outliers in both groups imply sporadic contamination events rather than isolated failures. Although no foodborne-disease outbreaks have been reported locally, this may reflect adaptive immunity among frequently exposed populations rather than genuine microbial safety.

From a public-health perspective, tolerance to subclinical exposure is not equivalent to safety—particularly for vulnerable groups such as infants, the elderly, pregnant women, and immunocompromised individuals. The wide 95 % confidence intervals extending well beyond the SNI limit (< 3 MPN/mL) indicate that population-level exposure to unsafe microbial loads remains a realistic risk.

To mitigate these hazards, a multi-tiered intervention strategy is essential:

Market-level microbial surveillance to enable early detection and removal of unsafe products.

Mandatory hygiene and sanitation training for both household-scale and industrial producers, promoting Good Manufacturing Practices (GMP) and Sanitation Standard Operating Procedures (SSOP).

Public-education programs to increase awareness

of safe storage, pasteurization, and handling.

Regulatory strengthening through systematic inspection, certification, and traceability enforcement. Comparative evidence supports these findings. Alang et al. (2024) observed substantial bacterial contamination in tempoyak (fermented durian paste) despite its traditional production, while Ezemba (2022) and Barmettler, Waser & Stephan (2025) reported similar E. coli contamination in soy-based drinks and fermented beverages across Africa. In contrast, studies from high-income countries consistently report pathogen-free soy milk, reflecting the impact of standardized pasteurization and stringent regulatory oversight.

This study demonstrates a serious public-health concern regarding *Escherichia coli* contamination in soy milk sold at Bandungan Traditional Market. Among the 30 samples analyzed, six samples (20%) were confirmed positive for E. coli, 20 samples (66.7%) exceeded the microbiological limit specified by SNI 7388:2009, and 10 samples (33.3%) complied with the standard. No statistically significant difference in contamination levels was observed between branded and unbranded soy milk (Mann–Whitney U = 70.5, p = 0.3024), indicating that microbiological contamination occurred across both product categories. These findings underscore the need for strengthened hygiene practices, routine microbiological surveillance, and quality assurance throughout the soy milk production and distribution chain.

Although no outbreaks or reported clinical cases of foodborne illness were documented, the absence of symptomatic infections does not imply safety. Reliance on population-level tolerance or adaptive immunity cannot substitute for preventive control. Chronic exposure to low-level E. coli contamination, even without immediate illness, poses cumulative health risks such as gastrointestinal disturbances and long-term alterations of gut microbiota composition.

To mitigate these risks, several urgent actions are needed. Hygiene management during production and distribution must be enhanced through the consistent application of Good Manufacturing Practices (GMP) and Sanitation Standard Operating Procedures (SSOP). Regulatory authorities should strengthen inspection, certification, and microbial surveillance systems to ensure compliance with national standards. Equally important, hygiene-education programs should be expanded to equip both small-scale producers and consumers with practical knowledge about food handling, pasteurization, and safe storage practices.

Safeguarding the microbiological safety of plant-based beverages is therefore a national public-health imperative. Effective protection will require coordinated efforts among regulatory agencies, producers, market vendors, and the public. Establishing a culture of food-safety awareness and accountability across all levels of the soy-milk supply chain is essential to prevent future contamination events and to protect vulnerable populations from foodborne disease.Safeguarding the microbiological safety of plant-based beverages is therefore a national public-health imperative. Effective protection will require coordinated efforts among regulatory agencies, producers, market vendors, and the public. Establishing a culture of food-safety awareness and accountability across all levels of the soy-milk supply chain is essential to prevent future contamination events and to protect vulnerable populations from foodborne disease.

3.3. Study Limitations

This study has several limitations. First, purposive sampling was used, with multiple samples collected from only five vendors at a single traditional market. Although each sample represented a separately purchased retail unit, information on production batches and production dates was not available; therefore, some degree of non-independence among observations cannot be ruled out. In addition, no formal sample size calculation or statistical power analysis was performed prior to sampling, as the number of samples was determined by product availability during the study period. Furthermore, product temperature at the time of purchase, vendor storage conditions, product shelf life, and display duration were not evaluated, although these factors may influence the microbiological quality of soy milk. Consequently, the findings should be interpreted with appropriate caution and may not be fully generalizable to all branded and unbranded soy milk products or other traditional market settings. Future studies should include a larger number of vendors, multiple market locations, samples from different production batches, probability-based sampling, an a priori sample size calculation, and the assessment of storage and handling conditions to improve sample independence, statistical power, and the generalizability of the findings.

4. CONCLUSIONS

This study identified an important food-safety concern regarding *Escherichia coli* contamination in soy milk sold at Bandungan Traditional Market. Of the 30 samples analyzed, six samples (20.0%) were confirmed positive for E. coli, while 13 samples (43.3%) exceeded the microbiological criterion of <3 MPN/mL established by SNI 7388:2009. The Mann–Whitney U test (U = 70.5, p = 0.3024) revealed no statistically significant difference in contamination levels between branded and unbranded soy milk. Therefore, the present findings do not support the conclusion that either product category has poorer microbiological quality. Instead, microbiological contamination should be regarded as a food-safety concern affecting both branded and unbranded soy milk products. These findings underscore the importance of strengthening hygiene practices,

routine microbiological surveillance, and quality assurance throughout the production and distribution chain to improve compliance with national food-safety standards and reduce the risk of microbiological contamination.

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REFERENCES

Alang, H. et al. (2024) ' Quality Analysis of Local Food Fermented Durian Paste (Tempoyak) in Pontianak Based on Nutrients and Bacterial Contaminants', Jurnal Pembelajaran Dan Biologi Nukleus, 10(3), pp. 784 – 795. DOI: 10.36987/jpbn.v10i3.6032

Barmettler, K., Waser, S. and Stephan, R. (2025) 'Microbiological quality of plant-based meat-alternative products collected at retail level in Switzerland', Journal of Food Protection, 88(1), p. 100402. https://doi.org/10.1016/j.jfp.2024.100402

Chandra, R. et al. (2022) 'Factors influencing consumer intention to purchase plant-based milk in Indonesia: an exploratory research', TIJAB (The International Journal of Applied Business), 6(2), pp. 145–164. https://doi.org/10.20473/tijab.v6.I2.2022.35257

Cui, J. (2025) 'A Comparative Analysis of Statistical Methods in Social Science Research: ANOVA, Multi-factor ANOVA, Chi-square, and t-test', ResearchGate. DOI: 10.13140/RG.2.2.36197.72164

Dakum, D. et al. (2022) 'Assistance in PIRT registration and halal certification for home industry Jenang Lot Karyasari, Bojong, Magelang Regency', Community Empowerment, 7(8), pp. 1453–1458. https://doi.org/10.31603/ce.6696

Ezemba, C.C. (2022) 'Assessment of Microbiological Quality of Soymilk Beverage', Available at SSRN 4085578 [Preprint]. DOI:10.56557/jogae/2022/v14i47690

Gao, Z. et al. (2024) 'Development and evaluation of a modified most probable number (MPN) method for enumerating rifampicin-resistant *Escherichia coli* in agricultural, food, and environmental samples', Journal of Food Safety, 44(3), p. e13127. DOI:10.1111/jfs.13127

Hashim, S., Rosli, N. and Addis, S.N.O.R.K. (2025) 'Microbiological assessment of soy milk collected from bazar Ramadhan in Kuala Nerus, Terengganu', Universiti Malaysia Terengganu Journal of Undergraduate Research, 7(1), pp. 1–7. DOI: https://doi.org/10.46754/umtjur.v7i1.395

He, Y. et al. (2025) 'Modified Most Probable Number Assay to Quantify Salmonella in Raw and Ready-to-Cook Chicken Products', Journal of Visualized Experiments (JoVE), (215), p. e67910. PMID: 39960189 DOI: 10.3791/67910

Kurnianto, M.F. et al. (2020) 'The Analysis of Consumer Perception on Quality of Soybean Milk Used Importance Performance Analysis Method', in IOP Conference Series: Earth and Environmental Science. IOP Publishing, p. 12051. DOI:10.1088/1755-1315/411/1/012051

Lirio, A.C.M. et al. (2023) 'Development of a Statistical Approach for Microbial Monitoring in Non-sterile Pharmaceutical Environments', Journal of Pharmaceutical Innovation, 18(3), pp. 1362–1370. DOI 10.1007/s12247-023-09719-4

Mahdizade Ari, M. et al. (2025) 'Viable but non-cultivable state in oral microbiota: a critical review of an underexplored microbial survival strategy', Frontiers in Cellular and Infection Microbiology, 15, p. 1533768. DOI: 10.3389/fcimb.2025.1533768

Manurung, K. A. et al. (2023) 'Comparative Test on the Level Contamination of *Escherichia coli* and Salmonella sp. on Broiler Meat at Slaughterhouses and Traditional Markets in Medan', Jurnal Peternakan Integratif, 11 (1), pp. 45–52. DOI : 10.32734/jpi.v11i1.11602

Mulyati, S. and Raharjo, M. (2024) 'Factors Associated with the Presence of E. coli in Food: Literature Review', Media Publikasi Promosi Kesehatan Indonesia (MPPKI), 7(7), pp. 1804–1810. DOI: https://doi.org/10.56338/mppki.v7i7.5325

Nisaa, R.K., Soedjoto, L. and Kunsah, B. (2020) 'Calculation of coliform number using most probable number (MPN) methods on soy milk sold in Pogot Area of Surabaya', Journal of SCRTE, 4(1), pp. 26–33. https://doi.org/10.20473/jscrte.v4i1.21591

Qin, P., Wang, T. and Luo, Y. (2022) 'A review on plant-based proteins from soybean: Health benefits and soy product development', Journal of Agriculture and Food Research, 7, p. 100265. https://doi.org/10.1016/j.jafr.2021.100265

Rahmanda, M., Feliatra, F. and Nursyirwani, N. (2025) 'Density of *Escherichia coli* Bacteria in Blood Clams (Anadara granosa) in Anak Setatah Village, Meranti Islands Regency, Riau', Jurnal Perikanan dan Kelautan, 30(2), pp. 212–218. DOI: https : //doi.org/10.31258/jpk.30.2.212-218

Riina, M.D. et al. (2023) 'Continuous variable analyses: T-test, Mann–Whitney, Wilcoxin rank', in Translational radiation oncology. Elsevier, pp. 153–163. https://doi.org/10.1016/B978-0-323-88423-5.00070-4

Roni, S.M. and Djajadikerta, H.G. (2021) Statistics and SPSS: Tests for Differences. Springer. DOI:10.1007/978-981-16-0193-4

Shakya, B., Shrestha, S.R. and Silvanus, V. (2021) 'Bacteriological Examination of Water supply and Drinking Water at Household level in Makalbari Area by the Most Probable Number Method', Nepal Medical College Journal, 23(2), pp. 146–152. DOI: https://doi.org/10.3126/nmcj.v23i2.38525

Tambi, A., Brighu, U. and Gupta, A.B. (2023) 'Methods for detection and enumeration of coliforms in drinking water: a review', Water Supply, 23(10), pp. 4047–4058. https://doi.org/10.2166/ws.2023.247

Utami, H.V.T. v(2023) 'Detection of Escherichia coli Bacterial Contamination on Cutlery at Warung Lesehan Around Nologaten Yogyakarta', Journal of Medical Laboratory in Infectious and Degenerative Diseases, 1(1), pp. 17–24. DOI: https://doi.org/10.36858/jmid.v1i1

Wardhana, D.K. et al. (2021) 'Detection of *Escherichia coli* contamination using most probable number (MPN) methods in chicken meats in market of Surabaya',

Jurnal Medik Veteriner, 4(1), pp. 118–124. https://doi.org/10.20473/jmv.vol4.iss1.2021.118-124

Warren, V., Bell, R. and Bruning-Mescher, S. (2024) 'Plant-based milk alternatives: Consumer needs and marketing strategies', in Plant-based food consumption. Elsevier, pp. 153–177. https://doi.org/10.1016/B978-0-323-98828-5.00006-1

Wolstenholme, R. and Forbes, S. (2021) 'Presumptive Testing', Analytical Techniques in Forensic Science, pp. 51–69. DOI:10.1002/9781119373421.ch3t