E-ISSN 2723-4959

Vol. 7 No. 2 December 2026

BIOFAAL JOURNAL



PATTIMURA UNIVERSITY

BIOFAAL JOURNAL

E-ISSN 2723-4959
Volume 7 Number 2 | December 2026

EDITOR IN CHIEF
Laury Marcia Ch. Huwae, S.Si., M.Si

Associate Editor
Efraim Samson, S.Si., M.Si
Abdul M Ukratalo, S.Si., M.Si
Edwin Thomas Apituley, S.Si.,M.Si

Expert Editorial Board
Prof. Dr. Pieter Kakisina, S.Pd., M.Si. (Pattimura University, Indonesia)
Prof. Dr. Dra. Adriana Hiariej, MP (Pattimura University, Indonesia)
Prof. Rajan Jose, Ph.D. (Ming Chi University of Technology, Taiwan)
Prof. Anom Bowolaksono, Ph.D. (University of Indonesia, Indonesia)
Prof. Dr. Pingkan Aditiawati, M.S (Bandung Institute of Technology, Indonesia)
Prof. Dr. Fenny Martha Dwivany, M.Si (Bandung Institute of Technology, Indonesia)
Prof. Sri Sumartiningsih, S.Si., M.Kes., Ph.D., AIFO (Semarang State University, Indonesia)
Dr. Handy Erwin Pier Leimena, S.Si., M.Si. (Pattimura University, Indonesia
dr. Laura B S Huwae, Sp.S., M.Kes. (Pattimura University, Indonesia)
Dr. dr. Ermita Isfandiary Ibrahim Ilyas, M.S., AIFO (University of Indonesia, Indonesia)

Asistant Editorial
Brian Saputra Manurung, S.Si., M.Sc
Kristi Lenci Patty, S.Si., M.Si
Fuadiska Salamena, S.Si., M.Si
Jecklyn Shindy Temartenan, S.Si., M.Biotech

Publisher
Department of Biology, Faculty of Science and Technology, Pattimura University,
in collaboration with
The Indonesian Association of Physiology Experts

Editorial Address
Department of Biology, Faculty of Science and Technology
Pattimura University
Jl. Ir. M. Putuhena, Poka Kampus, Kota Ambon, 97234
E-mail : biofaaljournal@gmail.com

BIOFAAL JOURNAL

Research Article

Article History:
Received : September 1, 2026
Revised : November 4, 2026
Accepted : November 11, 2026
Available online : November 14, 2026
Published : December 1, 2026

Key words:
Bakso tusuk, incubation time, total plate count

Corresponding Author:
Name: Nur Hikmah
Email: nurhik.mahhh20@gmail.com

Copyright © 2026 *Author(s)*
Publisher by Department of Biology, Faculty Science and Technology, Pattimura University.

Cite this article:
Hikmah, N., Safari, W.F. & Septiani. (2026), Effect of Incubation Time on the Total Plate Count of Bakso Tusuk (Indonesian Skewered Meatballs). *Biofaal Journal,* 7(2):110-122.
https://doi.org/10.30598/biofaal.v7i2pp110-122

Effect of Incubation Time on the Total Plate Count of Bakso Tusuk (Indonesian Skewered Meatballs)

Nur Hikmah[1*], Wulan Fitriani Safari[2], Septiani[3]

[1,2,3]Medical Laboratory Technology Study Program, Faculty of Health Sciences and Technology, Binawan University, Jakarta – Indonesia

Abstract
Bakso tusuk (Indonesian skewered meatballs) is a ready-to-eat food susceptible to microbial contamination due to open-air vending, poor sanitation, and inadequate storage. Total Plate Count (TPC) assesses microbiological quality based on Standar Nasional Indonesia (SNI) 3818:2014, which sets a maximum limit of 1×10^5 CFU/g. SNI 2897:2008 recommends an incubation period of 24–48 hours for TPC testing, which may affect bacterial colony recovery. This study evaluated the effect of incubation time on TPC results of bakso tusuk. An experimental study was conducted using three incubation periods (24, 36, and 48 hours) at 36°C. Samples were collected from four street vendors in East Jakarta. TPC analysis was performed using the pour plate method on Plate Count Agar (PCA). Colony counts generally increased with incubation time, reaching the highest values after 48 hours (1.1×10^9–3.8×10^{11} CFU/g). Sample D showed the greatest increase (1,390.57%) between 24 and 48 hours. Statistical analysis revealed an overall significant difference among incubation periods ($p = 0.008$), although not all pairwise comparisons were significant. All samples exceeded the SNI 3818:2014 limit. These findings suggest that incubation for up to 48 hours, as permitted by SNI 2897:2008, may improve bacterial colony recovery and reduce TPC underestimation.

INTRODUCTION

Bakso tusuk (Indonesian skewered meatballs) are one of the most widely consumed ready-to-eat foods because they are affordable, readily available, and convenient to eat. This product is commonly sold by street vendors in open environments, making it susceptible to microbial contamination from the air, equipment, raw materials, and food handlers (Mayaserli & Anggraini, 2019). Microbial contamination in food can reduce product quality and increase the risk of foodborne illnesses, such as diarrhea, nausea, vomiting, and food poisoning (Fitriani et al., 2023). The presence of pathogenic bacteria, including *Escherichia coli, Salmonella* spp., and *Staphylococcus aureus*, in meatball products has been widely reported in previous studies (Maulidina et al., 2023).

The Total Plate Count (TPC) method is one of the most commonly used techniques for evaluating the microbiological quality of food (Astuti et al., 2025) . This method determines the number of viable microorganisms capable of growing on culture media under specific incubation conditions. The TPC value serves as an indicator of hygiene, sanitation quality during food processing, and the microbiological safety of food products. An increase in the number of bacterial colonies indicates a higher level of microbial contamination in the product. The Indonesian National Standard (SNI) recognizes the TPC method as one of the standard

microbiological testing parameters for various food products, including processed meat products such as meatballs (Hartono et al., 2022). According to Indonesian National Standard (SNI) 3818:2014, the maximum permissible TPC for meatballs is 1×10^5 CFU/g, and products exceeding this limit are considered not to comply with the established microbiological quality standards.

Previous studies have reported microbial contamination in bakso tusuk (Indonesian skewered meatballs) marketed to the public. Mayaserli & Anggraini (2019) reported that one skewered meatball sample had a TPC value exceeding the maximum limit established by the Indonesian National Standard (SNI), with TPC values ranging from 2.3×10^3 to 1.5×10^5 CFU/g. Apriliyanti (2020) reported that several skewered meatball samples had TPC values ranging from 1.4×10^4 to 5.6×10^5 CFU/g, exceeding the permissible SNI limit, and tested positive for fecal indicator bacteria. Similarly, Hartono et al. (2021) reported that one of eight meatball samples collected from Makassar City had a TPC value of 3.09×10^5 CFU/g, which exceeded the maximum limit specified by the SNI. Consistent with these findings, Yanti et al. (2021) reported that some meatball products failed to meet microbiological quality standards based on their Total Plate Count values.

The accuracy of TPC analysis is influenced by several factors, including the type of culture medium Ughy et al. (2023), dilution technique, inoculation method Weitzel et al. (2021), incubation temperature, and incubation time. Incubation time determines the opportunity for bacteria to grow and form visible colonies. An incubation time that is too short results in a lower colony count because some bacteria have not yet reached their optimal growth phase. Conversely, an excessively long incubation period may cause colonies to overlap, merge, or enter the stationary and death phases, thereby affecting the accuracy of colony enumeration. Furthermore, SNI 2897:2008 recommends incubating PCA plates at 34–36°C for 24–48 h for the enumeration of aerobic mesophilic bacteria. This recommended incubation range allows laboratories to select any incubation period within 24 to 48 h according to their analytical procedures, although differences in incubation time may influence the number of recoverable bacterial colonies and, consequently, the reported TPC values. Therefore, selecting an appropriate incubation time is essential for obtaining accurate and representative TPC results Gonzalez & Aranda (2023).

Previous studies have generally used a single incubation time, either 24 or 48 hours, following standard testing procedures. Although these studies reported the microbiological quality of food samples, they did not examine how different incubation times affect bacterial colony counts. Since incubation time may influence colony recovery and the interpretation of microbiological quality, studies comparing different incubation times for the TPC of bakso tusuk remain limited. Therefore, this study compared three incubation times (24, 36, and 48 hours) to evaluate their effects on TPC results in bakso tusuk. This comparison is expected to provide scientific evidence regarding the influence of incubation duration on bacterial colony recovery and contribute to a better understanding of TPC analysis in accordance with SNI 2897:2008.

RESEARCH METHODS

The bakso tusuk samples consisted of skewered meatballs purchased from four street vendors at different locations in East Jakarta. Sample A was collected from a street vendor in the Kalibata area, in front of Kalibata City Apartment. Sample B was collected from a street vendor in the Kramat Jati area, opposite Kramat Jati Main Market. Sample C was collected from a street vendor in the Cibubur area, beside Al-Istiqomah Mosque. Sample D was collected from a street vendor in the Cililitan area, within the Cililitan Wholesale Center (PGC) on Mayjen Sutoyo Street. All samples were transported to the Microbiology Laboratory, Universitas Binawan, for TPC analysis.

Instruments and Materials

The equipment used in this study included an incubator, autoclave, laminar airflow cabinet, analytical balance, homogenizer blender, vortex mixer, micropipettes, sterile Petri dishes, test tubes, graduated cylinders, Erlenmeyer flasks, glass stirring rods, a Bunsen burner, a colony counter, and other standard laboratory glassware. The materials consisted of skewered meatball samples, Plate Count Agar (PCA), Buffered Peptone Water (BPW), sterile distilled water, 70% ethanol, and other supporting materials required for microbiological analysis.

Sample Preparation

Skewered meatball samples were purchased from street vendors at several locations in East Jakarta. The samples were placed in thin-wall containers that had been sterilized using 70% ethanol and dried with tissue. The samples were then transported to the Microbiology Laboratory of Binawan University using a cool box. Upon arrival, the samples were ground using a mortar and pestle. A total of 25 g of each sample was weighed aseptically for further microbiological analysis. Skewered meatball samples were purchased from street vendors at several locations in East Jakarta. The samples were placed in thin-wall containers that had been sterilized using 70% ethanol and dried with tissue. The samples were then transported to the Microbiology Laboratory of Binawan University using a cool box. Upon arrival, the samples were ground using a mortar and pestle. A total of 25 g of each sample was weighed aseptically for further microbiological analysis.

Total Plate Count (TPC) Analysis

The study began with the random collection of skewered meatball samples from four street vendors. Each sample was homogenized, followed by serial dilution using 0.1% BPW. One milliliter (1 mL) of the appropriate dilution was inoculated into a sterile Petri dish using the pour plate method. Sterile PCA was then added, and the contents were gently mixed to ensure even distribution of the inoculum. After the medium solidified, the plates were incubated at 36°C for 24, 36, and 48 hours. At the end of each incubation period, bacterial colonies were counted on plates containing colony numbers that met the counting criteria specified in SNI 2897:2008. The results were expressed as colony-forming units per gram (CFU/g) (Badan Standarisasi Nasional, 2008).

The TPC data were presented in tabular form and analyzed descriptively by comparing bacterial colony counts obtained at each incubation period. The analysis was performed to describe changes in TPC values after 24, 36, and 48 hours of incubation and to identify the incubation period that produced the most representative colony count (Badan Standarisasi Nasional, 2008).

Bacterial colonies were enumerated using a colony counter. Only plates containing 25–250 colonies were included in the analysis. The Total Plate Count (TPC) was calculated using the following formula:

$$N = \text{Number of colonies per plate} \times \frac{1}{Dilution\ factor}$$

Description:

N = Total Plate Count (CFU/g)

Data Analysis

Data were analyzed quantitatively based on the results of the TPC assay. Statistical analysis was performed using a repeated-measures analysis of variance (repeated-measures ANOVA) to evaluate differences in the mean TPC values among the three incubation periods. Prior to the main analysis, data normality was assessed using the Shapiro–Wilk test. A p-value > 0.05 indicated that the data were normally distributed, whereas a p-value < 0.05 indicated a non-normal distribution. If the normality assumption was not met, the Friedman test was used as the non-parametric alternative. A p-value < 0.05 was considered to indicate a statistically significant difference among the incubation periods. When significant differences were detected, Wilcoxon signed-rank tests with the Bonferroni correction were performed as post hoc analyses to identify which pairs of incubation periods differed significantly while controlling for multiple comparisons. The adjusted significance level was set at 0.0167 (0.05/3) (Eisinga et al., 2017).

RESULTS AND DISCUSSION

Total Plate Count (TPC) Values of Bakso Tusuk (Indonesian Skewered Meatballs) at Different Incubation Times

The Total Plate Count (TPC) values of the skewered meatball samples were determined based on the growth of bacterial colonies on PCA at each dilution level and incubation treatment. The results showed that the number of bacterial colonies decreased as the dilution factor increased. The colonies exhibited variations in both size and color. The number of colonies observed at each dilution served as the basis for calculating the TPC value for each sample. Representative bacterial colony growth on PCA following each incubation period is presented in Figure 1.

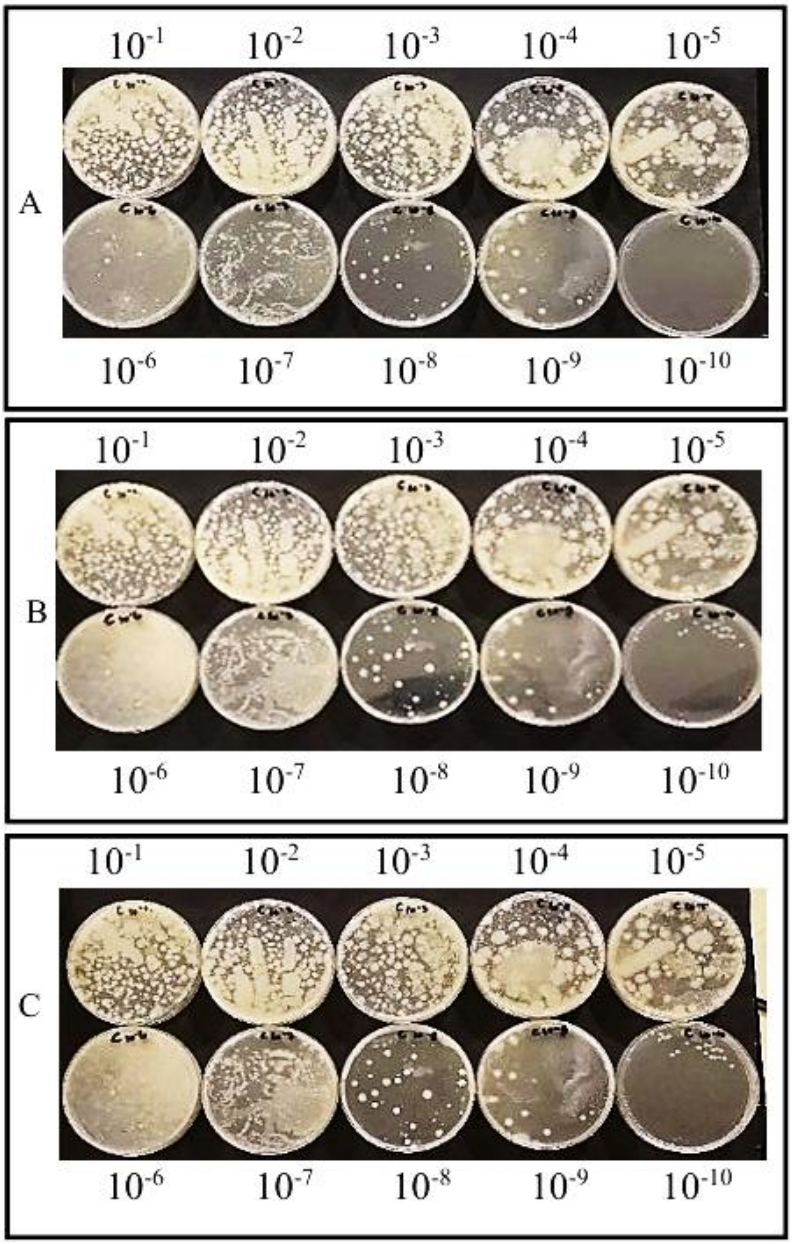


Figure 1. Bacterial colony growth on PCA from Bakso tusuk (Indonesian Skewered Meatballs) samples after incubation for (A) 24 hours, (B) 36 hours, and (C) 48 hours.

Figure 1. shows the bacterial colony growth on PCA from skewered meatball samples incubated for 24 hours (A), 36 hours (B), and 48 hours (C). The results demonstrated a progressive decrease in the number of colonies as the dilution level increased. This reduction occurred because the concentration of microorganisms decreased with each successive dilution step. The principle of serial dilution reduces the number of bacterial cells distributed onto the culture medium, resulting in fewer colonies developing on the agar surface (Laili et al., 2022).

The colonies exhibited considerable variation in color, size, and morphology. Differences in colony color are associated with the ability of bacteria to produce pigments during growth (Widianingrum et al., 2025). Variations in colony size are related to differences in bacterial growth rates and their ability to adapt to the culture medium (Zakaria & Jember, 2024). In

addition, the diversity of colony morphology indicates the presence of multiple microbial species within the samples. Different bacterial species exhibit distinct colony characteristics, including circular or irregular shapes and smooth or serrated margins (Zakaria & Jember, 2024). These observations suggest that the bacterial population present in the skewered meatball samples was microbiologically heterogeneous (Wardhani et al., 2020).

The Total Plate Count (TPC) values obtained at each dilution level for each treatment were used to calculate the TPC of each sample. The calculated values were then compared with the Indonesian National Standard (SNI) 3818:2014, which specifies a maximum permissible TPC limit of 1×10^5 CFU/g (Badan Standardisasi Nasional, 2014). The TPC values of the skewered meatball samples are presented in Table 1.

Table 1. Mean TPC values of four Bakso tusuk (Indonesian Skewered Meatballs) samples, each analyzed in triplicate, after incubation for 24, 36, and 48 hours.

Sample	Incubation Period	Mean TPC Value (CFU/g)	Interpretation
A	24 h	7.6×10^8	Exceeded the maximum permissible limit
	36 h	8.6×10^8	
	48 h	1.1×10^9	
B	24 h	3.52×10^{11}	Exceeded the maximum permissible limit
	36 h	3.7×10^{11}	
	48 h	3.8×10^{11}	
C	24 h	3.6×10^{10}	Exceeded the maximum permissible limit
	36 h	9.4×10^{10}	
	48 h	1×10^{11}	
D	24 h	5.3×10^9	Exceeded the maximum permissible limit
	36 h	1.0×10^{10}	
	48 h	7.9×10^{10}	

The analysis of differences between treatments was conducted to evaluate changes in bacterial colony counts under each incubation condition. Differences in TPC values among the incubation periods reflect the continuous growth of microorganisms during the incubation process. Greater increases in colony counts indicate that longer incubation periods provide bacteria with more time to grow and multiply. Table 2. presents the differences and Percentage Increase in TPC values between incubation treatments for all samples analyzed.

Table 2. Differences in TPC Values and Percentage Increase (%) Between the 24-, 36-, and 48-Hour Incubation Periods for Each bakso tusuk (Indonesian Skewered Meatballs) sample.

Sample	Difference in Mean TPC Value (CFU/g) & Percentage Increase (%)					
	24 h-36 h		36 h-48 h		24 h-48 h	
A	1.0×10^8	13.16 %	2.4×10^8	27.91 %	3.4×10^8	44.74 %
B	1.8×10^{10}	5.11 %	1.0×10^{10}	2.70 %	2.8×10^{10}	7.95 %
C	5.8×10^{10}	161.11 %	6.0×10^{10}	6.38 %	6.4×10^{10}	177.78 %

Sample	Difference in Mean TPC Value (CFU/g) & Percentage Increase (%)					
	24 h-36 h		36 h-48 h		24 h-48 h	
D	4.7×10^9	88.68%	6.9×10^{10}	690.00%	7.37×10^{10}	1390.57 %

Table 1. shows the that TPC values of all skewered meatball samples exceeded the maximum permissible microbial contamination limit specified by Indonesian National Standard (SNI) 3818:2014, which is 1×10^5 CFU/g (Badan Standardisasi Nasional, 2014). The TPC values obtained after 24 hours of incubation were higher than those reported in most previous studies. A study conducted in Gunung Talang District reported TPC values ranging from 2.3×10^3 to 1.5×10^5 CFU/g (Mayaserli & Anggraini, 2019), whereas a study in Gresik City reported values between 1.4×10^4 and 5.6×10^5 CFU/g (Apriliyanti, 2020). Another study conducted in Majene City reported a maximum TPC value of 5.8×10^6 CFU/g (Fitriani et al., 2023). These previous studies employed only a single incubation period and therefore described microbial contamination under only one testing condition. The incubation periods of 24, 36, and 48 hours resulted in a progressive increase in bacterial colony counts across all samples. The 36-hour incubation produced higher colony counts than the 24-hour incubation, while the 48-hour incubation yielded the highest TPC values for all samples.

Table 2. shows the that greatest increase in TPC values was observed between the 24-hour and 48-hour incubation periods. These findings indicate that extending the incubation period increases the number of viable bacteria capable of forming colonies on PCA. Based on the percentage increase, Sample D exhibited the highest increase of 1,390.57% during the 24–48 h incubation period, followed by Sample C (177.78%), Sample A (44.74%), and Sample B (7.95%). These differences in percentage increase indicate that bacterial growth responses varied among the samples. Samples with higher percentage increases suggest that the bacterial population remained in an active growth phase during incubation, whereas samples with lower percentage increases indicate that the bacterial population had become relatively stable or that the initial bacterial count was already high, resulting in a smaller relative increase. In addition, the 36 h incubation period provided additional information on the dynamics of bacterial colony growth, which has rarely been reported in previous studies.

The findings are consistent with a previous study conducted at Dramaga Traditional Market, Bogor, which reported an increase in TPC values when the incubation period was extended from 24 to 48 hours (Martiana & Padya, 2024). In the present study, the increase in TPC values ranged from 3.4×10^8 to 2.8×10^{11} CFU/g, indicating a substantially higher level of microbial contamination in the skewered meatball samples. These results suggest that inadequate sanitation, improper food handling, poor raw material quality, and prolonged environmental exposure during street vending may contribute to increased microbial contamination.

The increase in TPC values was influenced by the duration of incubation, the stability of the incubation temperature, and the availability of nutrients in the PCA medium. SNI 2897:2008 recommends an incubation period of 24–48 hours for the growth of mesophilic bacteria. A stable incubation temperature maintains bacterial enzymatic activity and metabolic processes, thereby promoting optimal colony development (Kurniawati et al., 2019). In

addition, PCA provides essential nutrients, including nitrogen, carbon, vitamins, minerals, and growth factors that support protein synthesis, cell division, and bacterial biomass production throughout the incubation period (Susilo, 2023).

The relatively high TPC values observed in the skewered meatball samples may also be associated with inadequate hygiene and sanitation during food preparation, handling, and storage. Potential sources of contamination include food handlers, processing equipment, water, containers, and exposure to the surrounding environment. Furthermore, the high moisture and nutrient content of meatballs may provide favorable conditions for bacterial growth, particularly when storage temperatures are not properly controlled. These factors may contribute to the high bacterial counts observed in the samples collected from street vendors in East Jakarta. However, differences in sampling procedures, sample characteristics, and analytical methods should also be considered when comparing these findings with previous studies conducted in other cities. Consequently, longer incubation periods may allow more bacterial colonies to develop and produce higher TPC values.

Effect of Incubation Time on the Total Plate Count (TPC) of Bakso Tusuk (Indonesian Skewered Meatballs)

The effect of incubation time on the TPC of bakso tusuk (Indonesian skewered meatballs) was evaluated using statistical analysis. Because the data did not meet the assumptions required for parametric testing, the Friedman test, a non-parametric test, was performed to assess differences among the three related incubation periods (24, 36, and 48 hours). The results of the analysis are presented in Table 3.

Table 3. Results of the Friedman Test for the Effect of Incubation Time on the TPC of Bakso tusuk (Indonesian skewered meatballs)

Incubation Period	Significance (p-value)
24 h	0.008
36 h	
48 h	

TPC values existed between individual pairs of incubation periods, a Wilcoxon signed-rank test was performed as a post hoc analysis. The level of statistical significance was adjusted using the Bonferroni correction by dividing the significance level ($\alpha = 0.05$) by the number of pairwise comparisons (three), resulting in an adjusted significance level of $\alpha = 0.0167$. The results of the pairwise comparisons are presented in Table 4.

Table 4. Results of the Wilcoxon Signed-Rank Test for Pairwise Comparisons of Incubation Periods

Comparison of Incubation Periods	p-value	Interpretation
24 h vs. 36 h	0.136	Not significant
24 h vs. 48 h	0.084	Not significant
36 h vs. 48 h	0.003	Significant

Note: Statistical significance was determined using the Bonferroni-adjusted significance level ($\alpha = 0.0167$). Only the comparison between 36 h and 48 h showed a statistically significant difference ($p < 0.0167$).

Table 3. the Friedman test demonstrated that incubation time significantly affected the TPC of the skewered meatball samples. Differences in TPC values reflect the dynamics of bacterial growth, which follows the typical bacterial growth curve consisting of the lag phase, exponential (log) phase, stationary phase, and death phase. Each growth phase produces different numbers of bacterial colonies, resulting in increased TPC values as the incubation period is extended. Mesophilic bacteria grow optimally at temperatures between 30°C and 37°C. Adequate nutrient availability, optimal temperature, and active bacterial metabolism promote bacterial proliferation and contribute to higher colony counts (Ali et al., 2023).

The 24-hour incubation period yielded relatively low colony counts because most bacteria were still transitioning from the lag phase to the exponential (log) phase. During this stage, bacterial cells adapt to the growth environment, synthesize enzymes, and produce essential cellular components before active cell division begins. After 36 hours of incubation, colony counts increased markedly as the bacteria entered the exponential phase, characterized by rapid metabolic activity and active cell division. At 48 hours, colony counts remained high, although the growth rate began to decline as the bacterial population entered the stationary phase. At this stage, nutrient depletion and the accumulation of metabolic by-products limited further growth, resulting in a balance between bacterial cell proliferation and cell death (Putra, 2023).

The findings indicate that variations in incubation time influence both bacterial colony counts and growth characteristics. Extending the incubation period allows bacterial populations to increase until optimal growth is reached, after which growth slows as the culture enters the stationary phase. These results are consistent with previous studies reporting increased growth of *Staphylococcus aureus* (Khelissa et al., 2017), *Bacillus licheniformis* (Oktavia et al., 2018), *Lactobacillus brevis* (Wahyu et al., 2020), and *Streptococcus mutans* with increasing incubation time until the optimal growth phase was attained (Widyawati & Situmeang, 2025).

Table 4. the Wilcoxon signed-rank test showed that not all pairwise comparisons between incubation periods resulted in statistically significant differences in TPC values (Wulandari et al., 2022). The comparison between 24 and 36 hours was not statistically significant, possibly because bacterial growth was still progressing and the differences in colony recovery had not yet become sufficiently consistent. In contrast, the comparison between 36 and 48 hours showed a statistically significant difference, indicating a change in bacterial colony recovery during the extended incubation period. Meanwhile, the comparison between 24 and 48 hours was not statistically significant, despite the longer incubation interval. This finding may be influenced by variations in bacterial growth patterns among samples, differences in colony count changes, and technical factors during serial dilution and colony enumeration. The relatively small sample size, consisting of only four street vendors, may also have limited the statistical power of the analysis. Therefore, the unexpected pattern

of pairwise differences should be interpreted cautiously and does not necessarily indicate that the longer incubation interval produces a smaller biological effect. Further studies involving a larger number of samples are needed to confirm these findings. Nevertheless, the incubation periods of 24–48 hours used in this study are consistent with the recommendations of SNI 2897:2008 for TPC analysis (Risma et al., 2022).

Increase in Colony Count with Increasing Incubation Time

The changes (Δ) and fold changes in TPC values were calculated based on different incubation periods. The calculations were performed for each comparison between incubation periods. The results are presented in Table 5.

Table 5. Changes (Δ) and Fold Changes in TPC Values Based on Incubation Time

Incubation Period Comparison	Δ (CFU/g)	*Fold Change*
24 h-36 h	2.02×10^{10}	1.67×
36 h-48 h	2.131×10^{10}	2.82×

The results presented in Table 5 indicate that TPC values increased during both incubation intervals. The mean fold change was 1.67-fold between 24 and 36 hours and 2.82-fold between 36 and 48 hours. The greater mean fold change during the 36–48-hour interval indicates a larger relative increase in TPC values during this period. This result was partly influenced by Sample D, which showed a particularly pronounced increase between 36 and 48 hours. These variations suggest that the magnitude of bacterial colony recovery differed among samples and incubation intervals. However, fold change values describe the relative magnitude of the observed changes and should not be interpreted independently as evidence of statistical or biological significance. Therefore, the fold change findings should be considered alongside the results of the statistical analysis.

CONCLUSION

This study demonstrated that incubation time significantly affected the overall TPC results of bakso tusu*k* (Indonesian skewered meatballs), as indicated by the Friedman test ($p < 0.05$). Although bacterial colony counts generally increased with longer incubation periods, the post-hoc analysis showed that not all pairwise comparisons were statistically significant. The highest TPC values were consistently obtained after 48 hours of incubation, indicating greater bacterial colony recovery than after 24 or 36 hours. Regardless of the incubation time, all samples exceeded the maximum permissible limit of 1×10^5 CFU/g specified in Standar Nasional Indonesia (SNI) 3818:2014, indicating unsatisfactory microbiological quality. These findings suggest that incubation time influences bacterial colony recovery and enumeration during TPC analysis. Therefore, within the incubation period permitted by SNI 2897:2008, incubation for up to 48 hours may provide greater bacterial colony recovery and reduce the potential underestimation of TPC values.

ACKNOWLEDGEMENTS

The authors would like to express their sincere gratitude to the Microbiology Laboratory of Binawan University for providing the research facilities that made this study possible.

DECLARATIONS

Author Contributions

N.H conceptualized and designed the study, conducted sample collection and processing, performed the laboratory experiments, analyzed and interpreted the data, and prepared the manuscript. W.F.S supervised the study design, provided input on the data analysis, and reviewed and revised the manuscript. S supervised the research implementation, validated the research findings, and reviewed and revised the manuscript. All authors have read and approved the final version of the manuscript.

Funding

This research received no financial support from government agencies, private institutions, or non-profit organizations. All research activities were self-funded by the authors.

Ethical Statement

This study did not involve human participants or animals as research subjects; therefore, ethical approval from an institutional review board or ethics committee was not required. All research procedures were conducted in accordance with established laboratory standard operating procedures and accepted scientific principles.

Declaration of Interest

The authors declare that they have no financial or non-financial conflicts of interest that could have influenced the conduct of this study or the interpretation of its findings.

Data Sharing Statement

The datasets generated and analyzed during this study are included in this published article. Additional data supporting the findings of this study are available from the corresponding author upon reasonable request.

REFERENCE

Ali, M. A., Hashish, M. H., & Fekry, M. M. (2023). Microbiological quality of some packed and unpacked bread products in Alexandria, Egypt. Journal of the Egyptian Public Health Association, 98, 1–9. https://doi.org/10.1186/s42506-023-00141-9

Apriliyanti, L. D. (2020). Analisis kandungan mikroba pada jajanan bakso tusuk di Alun-Alun Kota Gresik menggunakan metode TPC (Total Plate Count) dan MPN (Most Probable Number). [Skripsi]. Universitas Islam Negeri Sunan Ampel Surabaya

Astuti, R. A. W., Lestari, N. F., Aprianie, W., & Hidayati, L. (2025). Perbandingan metode *pour plate*, *spread plate* dan *drop plate* pada pemeriksaan *Total Plate Count* (TPC) minuman

tebu (*Saccharum officinarum* L.). *Jurnal Analis Kesehatan, 15*(1). https://doi.org/10.26630/jak.v15i1.5681

Eisinga, R., Heskes, T., Pelzer, B., & Te Grotenhuis, M. (2017). Exact p-values for pairwise comparison of Friedman rank sums, with application to comparing classifiers. BMC Bioinformatics, 18, Article 68. https://doi.org/10.1186/s12859-017-1486-2

Fitriani, F., Said, N. S., & Syah, S. P. (2023). Identifikasi cemaran mikroba pada jajanan bakso di Kota Majene. Jurnal Riset Rumpun Ilmu Hewani, 2(1), 22–36. https://doi.org/10.55606/jurrih.v2i1.1330

Gonzalez, J. M., & Aranda, B. (2023). Microbial growth under limiting conditions: Future perspectives. Microorganisms, 11(7), 1641. https://doi.org/10.3390/microorganisms11071641

Hartono, H., Ali, A., Idris, I. S., & Daud, H. I. (2021). Cemaran bakteri pada bakso yang beredar di Kota Makassar. Indonesian Journal of Fundamental Sciences, 7(2), 84–91. https://doi.org/10.26858/ijfs.v7i2.26154

Khelissa, S. O., Jama, C., Abdallah, M., Boukherroub, R., Faille, C., & Chihib, N. E. (2017). Effect of incubation duration, growth temperature, and abiotic surface type on cell surface properties, adhesion and pathogenicity of biofilm-detached Staphylococcus aureus cells. AMB Express, 7, 200. https://doi.org/10.1186/s13568-017-0492-0

Kurniawati, L., Kusdiyantini, E., & Wijanarka. (2019). Pengaruh variasi suhu dan waktu inkubasi terhadap aktivitas enzim selulase dari bakteri Serratia marcescens. Jurnal Akademika Biologi, 8(1), 1–9.

Laili, N. H., Abida, I. W., Junaidi, A., et al. (2022). Nilai Total Plate Count (TPC) dan jumlah jenis bakteri air limbah cucian garam (bittern) dari tambak garam Desa Banyuajuh Kecamatan Kamal Kabupaten Bangkalan. Juvenil: Jurnal Ilmiah Kelautan dan Perikanan, 3(1), 26–31. https://doi.org/10.21107/juvenil.v3i1.15075

Martiana, A., & Padya, I. R. (2024). Analisis cemaran mikroba bakso di Pasar Tradisional Dramaga, Bogor. Jurnal Ilmiah Betahpa, 3(1), 1–7. https://doi.org/10.36050/9yhyv159

Maulidina, R., Marlina, E. T., & Utama, D. T. (2023). Mutu mikrobiologi produk olahan daging yang dijual secara daring dari UMKM di Kota Bandung. Jurnal Teknologi Hasil Peternakan, 4(2), 83–100. https://doi.org/10.24198/jthp.v4i2.47313

Mayaserli, D. P., & Anggraini, D. (2019). Identifikasi bakteri Escherichia coli pada jajanan bakso tusuk di Sekolah Dasar Kecamatan Gunung Talang Tahun 2018. Jurnal Kesehatan Perintis, 6(1), 30–34. https://doi.org/10.33653/jkp.v6i1.220

Oktavia, Y., Lestari, S. D., Lestari, S., Herpandi, & Jannah, M. (2018). Optimasi waktu inkubasi produksi protease dan amilase isolat bakteri asal terasi ikan teri (Stolephorus sp.). Jurnal Ilmu dan Teknologi Kelautan Tropis, 10(3), 719–726. https://doi.org/10.29244/jitkt.v10i3.18840

Putra, S. G. (2023). Gangguan kesehatan pada masyarakat yang disebabkan oleh bakteri mesofilik. Jurnal Pengabdian Masyarakat Biologi dan Sains. https://doi.org/10.30998/jpmbio.v2i2.2376

Risma, Y. K., Harimurti, S., Wihandoyo, & Widodo. (2022). Kurva pertumbuhan isolat bakteri asam laktat dari saluran pencernaan itik lokal asal Aceh. Jurnal Peternakan Indonesia, 24(1), 1–7. https://doi.org/10.25077/jpi.24.1.1-7.2022

Susilo. (2023). Mikrobiologi Dasar. Ahlimedia Press

Ughy, B., Nagyapati, S., Lajko, D. B., Letoha, T., Prohaszka, A., Deeb, D., et al. (2023). Reconsidering dogmas about the growth of bacterial populations. Cells, 12(10), 1430. https://doi.org/10.3390/cells12101430

Wahyu, A., Indah, N., & Rahayu, W. P. (2020). Optimum growth conditions of Lactobacillus brevis LIPI13-2-LAB131 in β-galactosidase enzyme production. Biodiversitas, 21(11), 5403–5407. https://doi.org/10.13057/biodiv/d211147

Wardhani, A. K., Uktolseja, J. L. A., & Djohan, D. (2020). Identifikasi morfologi dan pertumbuhan bakteri pada cairan terfermentasi silase pakan ikan. Prosiding Seminar Nasional Pendidikan Biologi dan Saintek, 411–419.

Weitzel, M. L. J., Vegge, C. S., Pane, M., Goldman, V. S., Koshy, B., Porsby, C. H., et al. (2021). Improving and comparing probiotic plate count methods by analytical procedure lifecycle management. Frontiers in Microbiology, 12, 693066. https://doi.org/10.3389/fmicb.2021.693066

Widianingrum, D. C., Tanzil, A. I., Masnilah, R., & Nurcahyanti, S. D. (2025). Skrining dan identifikasi bakteri asam laktat dari rumen ternak sebagai kandidat probiotik. Jurnal Sain Veteriner, 43(2), 369–377. https://doi.org/10.22146/jsv.104307

Widyawati, & Situmeang, B. (2025). The effect of glucose on Streptococcus mutans ATCC 25175 growth by variation of concentration and incubation time. Pharmacoscript, 8(2), 395–403. https://doi.org/10.36423/pharmacoscript.v8i2.2179

Wulandari, E., Putranto, W. S., Gumilar, J., Suryaningsih, L., Pratama, A., & Anggaini, T. K. (2022). Kecepatan pertumbuhan spesifik bakteri asam laktat dengan ekstrak kacang merah (Phaseolus vulgaris L.) sebagai studi awal produksi flavored yogurt. Jurnal Agripet, 22(1), 72–78. https://doi.org/10.17969/agripet.v22i1.21129

Yanti, N. P. Y. C., Sudarmanto, I. G., & Sarihati, I. G. A. D. (2021). Gambaran angka lempeng total dan identifikasi Escherichia coli pada bakso ayam yang dijual di Desa Sanur Kauh Denpasar Selatan. The Journal of Medical Laboratory, 9(2), 129–137. https://doi.org/10.33992/m.v9i2.1746

Zakaria, M. A., & Jember, P. N. (2024). Pertumbuhan koloni bakteri dan karakteristik morfologi bakteri akar tebu dalam cairan terfermentasi. Jurnal Ilmu Pertanian, 1(1), 29–43. https://doi.org/10.71333/nhevp105