



Microbiological Stability of Traditional Rendang Boleces Prepared from Spent Hen Meat Under Ambient Storage Conditions

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Abstract. *Spent hen meat has potential as an alternative raw material for traditional ready-to-eat meat products; however, its microbiological quality during storage requires evaluation. This study assessed the microbiological quality of rendang boleces (a meatball-like product) prepared from spent hen meat using Total Plate Count (TPC) analysis and Salmonella spp. detection. Samples were stored under ambient conditions (25–28°C) for 0, 2, 4, and 6 days. TPC values increased during storage, from 2.67 log CFU/g on day 0 to 4.63 log CFU/g on day 6. Samples stored for up to two days remained within the maximum permissible limit established by the Indonesian National Standard (SNI). In contrast, higher microbial counts were observed on days 4 and 6, indicating progressive microbial growth during extended storage. Salmonella spp. was not detected in any sample throughout the observation period. These findings indicate that rendang boleces maintained acceptable microbiological quality for up to two days under ambient storage conditions. The study also highlights the importance of appropriate storage and hygienic handling practices to maintain the quality of ready-to-eat meat products derived from spent hen meat while supporting the sustainable utilization of poultry resources in line with SDG 12 (Responsible Consumption and Production).*

Keywords: *ambient storage; rendang boleces; salmonella spp; spent hen meat; total plate count.*

Type of the Paper: Regular Article.



1. Introduction

Spent laying hens, commonly referred to as spent hens, are poultry that have reached the end of their egg-laying cycle and represent a substantial by product of the poultry industry [1]. Despite their high protein content, spent hens are generally underutilized due to their tougher meat texture and lower consumer acceptance compared with broiler chickens [2]. Nevertheless, spent hen meat has potential for use in processed meat products, particularly traditional ready-to-eat foods, as an alternative strategy to improve the utilization of low-value poultry resources [1]. Previous studies have demonstrated that processing technologies can enhance the functional and sensory properties of spent hen meat, thereby increasing its potential for food product diversification [2–4].

One promising valorization strategy is the development of ready-to-eat (RTE) products derived from spent hen meat, such as rendang boleces. In this study, the term “boleces” refers to a modified traditional Indonesian rendang prepared from spent hen meatballs as the primary protein source. Unlike conventional rendang, which generally uses intact meat cuts, this product utilizes processed spent hen meatballs incorporated into traditional rendang seasoning and subjected to extended thermal processing [1]. This approach offers an alternative utilization of low-value poultry resources and influences the microbiological quality and storage stability of the final product due to differences in composition and processing characteristics.

Despite its potential application in ready-to-eat products, the utilization of spent hen meat requires careful microbiological evaluation. Compared with broiler meat, spent hens may carry higher microbial loads due to longer rearing periods and handling conditions [2]. In ready-to-eat meat products, microbial growth during storage may reduce product quality and increase the risk of spoilage, particularly under ambient tropical conditions. Total Plate Count (TPC) is widely used as an indicator of overall microbial quality and hygienic status in processed foods [5]. According to the Indonesian National Standard (SNI), the maximum permissible TPC for cooked ready-to-eat meat products is 1×10^4 CFU/g [6]. Therefore, monitoring microbial changes during storage is important to evaluate the microbiological quality and shelf life of rendang boleces.

In addition to the overall microbial load, the presence of foodborne pathogens such as *Salmonella spp.* remains a major concern in poultry-based products. *Salmonella spp.* is commonly associated with raw and processed poultry meat and is one of the leading causes of foodborne illness worldwide [7]. Contamination may occur during slaughter, processing, handling, or storage, particularly under inadequate hygienic conditions [8]. Although intensive thermal processing during rendang preparation may reduce microbial contamination, post-processing contamination and microbial survival during storage remain potential risks. Therefore, the detection of *Salmonella spp.* is important to evaluate the microbiological quality of rendang boleces during ambient storage.

Although the microbiological quality of poultry-based foods has been widely investigated, most previous studies have focused on broiler-based products or industrially processed foods stored under refrigerated conditions [9–11]. Limited information is available on the microbiological stability of traditional ready-to-eat foods prepared from spent hen meat and stored under ambient tropical conditions. Previous studies reported that microbial loads in poultry products may increase rapidly during storage, even under controlled packaging or refrigeration systems [12,13]. However, microbiological evaluation of traditionally processed spent hen products under ambient storage conditions remains limited. This information gap restricts the

availability of scientific data needed to evaluate the microbiological quality and storage stability of such products under realistic household and small-scale food processing conditions [14].

Therefore, this study aims to evaluate the microbiological quality of rendang boleces prepared from spent hen meat during ambient storage using Total Plate Count (TPC) analysis and *Salmonella spp.* detection. Unlike previous studies that mainly focused on refrigerated or industrially processed poultry products, this study evaluates a traditionally processed, spent hen-based ready-to-eat product stored under open ambient conditions commonly encountered in household and small-scale food systems. The findings are expected to provide scientific information on the microbiological quality and storage stability of rendang boleces during storage while supporting the sustainable utilization of poultry resources in line with SDG 12 (Responsible Consumption and Production).

2. Materials and Methods

2.1. Study Location

The study was conducted at the Culinary Workshop, Universitas Negeri Padang, Indonesia, and at the Agricultural Product Technology Laboratory, Universitas Andalas, Padang, Indonesia.

2.2. Kitchen Equipment Preparation

Product preparation was conducted using standard kitchen equipment under controlled procedures to ensure process consistency and minimize batch variability [15]. Ingredients were prepared and homogenized using a food processor (Philips, Netherlands), while ingredient quantities were measured using calibrated digital scales and measuring spoons to ensure accuracy [16]. Cooking was performed using stainless steel and carbon steel cookware on an electric cooktop under controlled heating conditions to support uniform heat distribution and hygienic processing [17].

2.3. Preparation of Spent Hen Meatballs

Spent hen meatballs were prepared according to the method described by Sari et al. [18]. A total of 500 g of spent hen meat was used as the primary protein source. The formulation consisted of carrageenan (12 g), tapioca flour (80 g), wheat flour (8 g), egg white (33 g), garlic (30 g), shallots (30 g), salt (5 g), sugar (2.5 g), ground pepper (2.5 g), lime juice (30 g), ice cubes (80 g), and spring onions (10 g) [19–22]. The meat was finely ground with ice cubes, followed by the addition of flour and seasonings until a homogeneous dough was obtained. The dough was shaped into uniform balls using two spoons and boiled in water for 15 minutes until fully cooked [23]. The cooked meatballs were then drained and cooled before further processing [24].

2.4. Preparation of Rendang Boleces

Rendang boleces was prepared according to a modified method from previous studies [25]. A total of 1000 g of spent hen meatballs and 1000 mL of fresh coconut milk were used as the main ingredients. The spice mixture consisted of shallots, garlic, red chili, ginger, galangal, lemongrass, bay leaves, orange leaves, nutmeg, turmeric leaves, ground coriander, salt, and cooking oil [26,27].

All ingredients were combined in a pan and cooked at 90–93°C for 90 minutes until the mixture thickened. The spent hen meatballs were then added and simmered until the product reached the *kalio* stage (wet rendang). To obtain dry rendang, the temperature was reduced to 80–83°C, and cooking was continued for approximately 2 hours with regular stirring until the product turned dark brown to black. The cooked rendang boleces was cooled before microbiological analysis [28].

2.5. Preparation of Microbiological Analysis Equipment

Microbiological analyses, including Total Plate Count (TPC) and detection of *Salmonella spp.*, were performed using standardized methods under aseptic conditions to ensure reproducibility [29]. Samples were weighed using an analytical balance and homogenized in sterile stomacher bags to obtain uniform microbial suspensions [30]. Serial dilutions were prepared using sterile pipettes, volumetric glassware, calibrated micropipettes, and vortex mixing to ensure homogeneous suspensions [31]. Culture preparation and inoculation procedures were conducted in a Class II Type A2 biosafety cabinet to maintain aseptic conditions [32]. Incubation was performed using a digital incubator, and the colony was enumerated using a digital colony counter [29]. All equipment and media were sterilized before analysis [30].

2.6. Microbiological Analysis Procedures

Rendang boleces were stored under ambient open air conditions (25–28°C) without reheating or additional preservation treatment for up to six days. Microbiological analyses were conducted at four storage intervals (0, 2, 4, and 6 days). Microbiological quality was evaluated using Total Plate Count (TPC) and detection of *Salmonella spp.* as primary food safety indicators [33,34]. TPC analysis was used to assess the overall microbial load during storage, while *Salmonella spp.* detection was performed because of its association with poultry-based products, including spent hen meat [35,36]. Since rendang boleces is intended for ready-to-eat or minimally reheated consumption, microbiological monitoring during ambient storage was considered important to evaluate product quality and safety [37].

2.6.1. Total Plate Count (TPC)

Total Plate Count (TPC) analysis was performed following standard microbiological procedures [38]. A 10 g sample was aseptically homogenized in 90 mL of buffered peptone water using a laboratory stomacher. Serial tenfold dilutions were prepared, and 1 mL aliquots were inoculated onto Plate Count Agar (PCA) using the pour plate method. The plates were incubated

at $30 \pm 1^\circ\text{C}$ for 72 ± 3 h, and colonies within the countable range of 30–300 CFU were enumerated. Results were expressed as log CFU/g [39]. TPC analysis was used as an indicator of overall microbial quality during storage. Although advanced microbial detection methods have been developed, conventional TPC analysis remains widely applied in food microbiology studies [40].

2.6.2. Detection of *Salmonella* spp.

Detection of *Salmonella* spp. was performed using standard culture-based methods [40]. A 25 g sample was aseptically pre-enriched in 225 mL of buffered peptone water and incubated at $37 \pm 1^\circ\text{C}$ for 18-24 hours. Selective enrichment was subsequently conducted using Rappaport Vassiliadis Soy (RVS) broth ($42 \pm 1^\circ\text{C}$, 24 h) and Müller Kauffmann Tetrathionate Novobiocin (MKTn) broth ($37 \pm 1^\circ\text{C}$, 24 h) [41]. Enriched cultures were streaked onto Xylose Lysine Deoxycholate (XLD) and Bismuth Sulfite Agar (BSA) plates and incubated at $37 \pm 1^\circ\text{C}$ for approximately one to two days. Suspected colonies were confirmed using Triple Sugar Iron (TSI), Lysine Iron Agar (LIA), Sulfide Indole Motility (SIM) tests, and Gram staining [42].

2.7. Data Analysis

All analyses were performed in triplicate, and quantitative data are presented as mean $\pm$ standard deviation. One-way analysis of variance (ANOVA), followed by Tukey's post hoc test, was used to evaluate differences among storage periods at a significance level of $p < 0.05$. Descriptive analysis was applied when no detectable colonies were observed. Detection results of *Salmonella* spp. were interpreted qualitatively based on the presence or absence of suspected colonies. All statistical analyses were performed using SPSS Statistics version 29.

3. Results and Discussion

3.1. Total Plate Count (TPC)

Total Plate Count (TPC) values of rendang boleces formulated from spent hen meat during ambient open storage are presented in Table 1. The initial microbial load on day 0 was 2.67 ± 0.05 log CFU/g and slightly increased to 2.93 ± 0.03 log CFU/g on day 2; with both values remained below the maximum permissible limit established by the Indonesian National Standard (SNI) for cooked meat products and the generally accepted international limit for ready-to-eat meat products [43,44]. A significant increase ($p < 0.05$) in TPC was observed on day 4 (4.23 ± 0.07 log CFU/g) and day 6 (4.63 ± 0.09 log CFU/g), indicating progressive microbial proliferation during storage. Changes in the physical appearance of rendang boleces during storage are presented in Fig. 1 and aligned with the observed increase in microbial load. Based on these findings, rendang boleces maintained microbiological quality within the acceptable SNI limit for up to two days under ambient open-air storage conditions without refrigeration or reheating.

The increase in TPC during storage reflects active microbial growth commonly observed in thermally processed meat products stored under ambient conditions without additional preservation treatments. The combination of meat proteins, coconut milk, and residual moisture may provide favorable conditions for microbial proliferation during storage. In addition, heat-resistant spore-forming bacteria that survive thermal processing may contribute to microbial growth during prolonged storage [45]. Similar increases in microbial counts during ambient storage have been reported in ready-to-eat meat products and poultry-based food lacking refrigeration or preservative systems [46,47].

Table 1. Total Plate Count (TPC) results and statistical analysis of rendang boleces during storage.

Storage time (day)	P-value	Total plate count (log CFU/g)	SNI Microbial Limit (log CFU/g)	Compliance Status
0	<0.001**	2.67 ± 0.05^a	4	Compliant
2		2.93 ± 0.03^a	4	Compliant
4		4.23 ± 0.07^b	4	Non-compliant
6		4.63 ± 0.09^b	4	Non-compliant

Note: Values followed by the same lowercase letter are not significantly different, while different lowercase letters indicate significant differences at $p < 0.05$ based on Tukey's post hoc test. The maximum acceptable limit for Total Plate Count is based on the Indonesian National Standard (SNI) for cooked meat products.

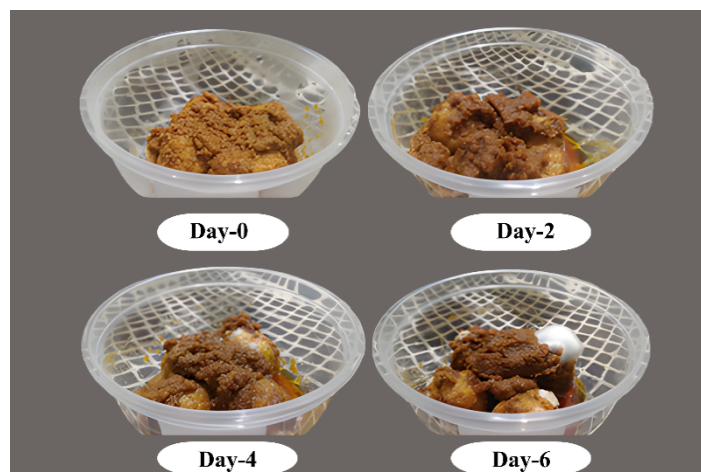


Fig. 1. Changes in the physical appearance of rendang boleces during storage.

Visible mold growth observed on day 4 (Fig. 1) may indicate fungal proliferation under ambient storage conditions, despite TPC values remaining relatively close to the SNI threshold. This condition may occur because TPC analysis primarily reflects total aerobic bacterial populations and does not specifically quantify fungal growth. The combination of residual moisture, coconut milk, oxygen exposure, and ambient temperature may create favorable conditions for mold development on the product surface during storage. Therefore, visual spoilage characteristics should be considered alongside microbiological standards when evaluating product quality and shelf life.

The progressive increase in microbial load also indicates that ambient storage conditions may accelerate spoilage in rendang boleces because of continuous exposure to oxygen,

temperature fluctuations, and nutrient availability within the product matrix. Previous studies demonstrated that antimicrobial packaging systems and natural preservative compounds could delay microbial growth in meat products stored under non-refrigerated conditions [48]. Therefore, the application of refrigeration, improved packaging systems, or natural antimicrobial ingredients may represent potential strategies to enhance the microbiological stability and shelf life of rendang boleces prepared from spent hen meat [27,46]. Although spent hen meat represents a potential alternative protein source, its utilization in ready-to-eat products requires careful microbiological control because spent hens may carry higher initial microbial loads than younger broilers [49]. Therefore, appropriate processing and storage strategies are important to maintain product quality and consumer safety during storage.

3.2. Identification of *Salmonella* spp.

The results of *Salmonella* spp. detection in rendang boleces prepared from spent hen meat during ambient storage is presented in Table 2. *Salmonella* spp. was not detected in any sample throughout the storage period (days 0, 2, 4, and 6). These findings comply with the Indonesian National Standard (SNI 7388:2009) and the Regulation of the National Agency of Drug and Food Control (PerBPOM) No. 3 of 2019, which require the absence of *Salmonella* spp. in 25 g of ready-to-eat meat products [6,43].

Table 2. *Salmonella* spp. detection results in spent hen meat rendang boleces during storage.

Day	Detected Colonies	SNI Microbial Standard	Compliance Status
0	0	Negative	Compliant
2	0	Negative	Compliant
4	0	Negative	Compliant
6	0	Negative	Compliant

Note: According to the Indonesian National Standard (SNI 7388: 2009), the presence of *Salmonella* spp. in ready-to-eat meat products must be undetectable in 25 g of sample.

The absence of *Salmonella* spp. throughout storage may be associated with the intensive thermal processing applied during rendang preparation. Prolonged cooking at high temperatures may effectively reduce pathogenic bacterial populations, including *Salmonella* spp. [50,51]. In addition, spices such as garlic, turmeric, and lemongrass contain bioactive compounds with reported antimicrobial activity that may contribute to inhibiting bacterial survival during storage [52]. Similar findings have been reported in thermally processed meat products containing natural spices and herbs, in which microbial inhibition was associated with the combined heat treatment and antimicrobial phytochemicals [53,54].

Although *Salmonella* spp. was not detected during storage, the progressive increase in TPC values and the appearance of visible mold growth on day 4 indicate that microbiological deterioration still occurred under ambient storage conditions. This suggests that the absence of *Salmonella* spp. alone should not be used as the sole indicator of overall product quality during

prolonged storage. Therefore, appropriate storage and handling practices remain important to maintain the microbiological quality of rendang boleces during distribution and consumption [55,56].

4. Conclusions

The present study demonstrated that rendang boleces prepared from spent hen meat maintained acceptable microbiological quality for up to two days under open ambient storage conditions, as indicated by Total Plate Count (TPC) values within the permissible Indonesian National Standard (SNI) limit and the absence of *Salmonella spp.* throughout the observation period. Progressive microbial growth observed after day 2 indicated microbiological deterioration during prolonged ambient storage, highlighting the importance of appropriate storage and hygienic handling practices for ready-to-eat meat products. This study was limited to microbiological evaluation under ambient storage conditions and did not include sensory, fungal, or refrigerated storage analyses. Therefore, further studies involving extended storage systems, packaging technologies, and additional quality parameters are recommended to improve product stability and safety. The utilization of spent hen meat in traditional ready-to-eat products also supports sustainable poultry resource management in line with SDG 12 (Responsible Consumption and Production).

Abbreviations

TPC	Total Plate Count
CFU	Colony Forming Unit
SNI	Indonesian National Standard
SDG	Sustainable Development Goals
RTE	Ready To Eat
PCA	Plate Count Agar
RVS Broth	Rappaport vassiliadis soya peptone broth
MKKTn Broth	muller kauffmann tetrathionate novobiocin broth
XLD Agar	Xylose Lysine Deoxycholate Agar
BSA	Bismuth Sulfite Agar
TSI	Triple Sugar Iron
LIA	Lysine Iron
SIM	Sulfide Indole Motility Medium
ANOVA	Analysis Of Variance
SPSS	Statistical Package For The Social Sciences

Data availability statement

The data supporting this study are available from the authors upon reasonable request.

Credit authorship contribution statement

Rahmi Holinesti: was responsible for developing the fundamental research concept, supervising the research activities, and preparing the publication manuscript. **Anni Faridah:** contributed to the design and implementation of the research methodology. **Mimi Harni:**

contributed to the discussion of the research findings. **Noveria Sjafrina** and **Sari Mustika** were responsible for the total plate count (TPC) and shelf life analyses, as well as the detection and analysis of *Salmonella spp.*, while **Ranggi Rahimul Insan** conducted the statistical analysis and data interpretation.

Declaration of Competing Interest

The authors of this manuscript declare no conflict of interest or competing interest.

Declaration of Use of AI in the Writing Process

The authors used ChatGPT during the preparation of this manuscript to improve grammar, paraphrase text, and enhance clarity. After using the tool, the authors carefully reviewed and edited the content and take full responsibility for the content of the publication.

Acknowledgement

The authors sincerely thank all colleagues, laboratory staff, and technical personnel who actively contributed to the completion of this study through their expertise, guidance, and hands on assistance. Their invaluable support greatly facilitated the research process.

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