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Microbiological and quality characteristics of chicken and beef meatballs under two hygienic protocols during refrigerated storage

Atchara Nuphet Phoem[1*], Supranee Phakul[1], Aroonrat Phoem[2]

[1]Bachelor of Science Program in Biotechnology, Faculty of Science and Technology, Songkhla Rajabhat University, Songkhla, Thailand.
[2]Independent Researcher, Songkhla, Thailand.

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ABSTRACT

This descriptive study compared chicken and beef meatballs produced under conventional and modified hygienic protocols during refrigerated storage at 4°C. For each meat type, one raw-material lot was divided between protocols, and each combination was manufactured once. The modified protocol combined enhanced sanitation and personnel hygiene, crushed ice during mixing, and 10-minute cold-water cooling; both protocols used vacuum packaging. Total viable count (TVC) and presumptive foodborne bacterial counts were assessed using laboratory-modified culture procedures without confirmatory testing. TVC increased during storage, remained below the study-selected comparative threshold of 4 log CFU/g through Day 18, and exceeded it on Day 24, with similar values between protocols. Presumptive *Staphylococcus aureus* counts were numerically lower in modified runs, mostly below the quantification limit; presumptive *Bacillus cereus* counts were similar between protocols and reached 2.80–2.91 log CFU/g on Day 24. No presumptive *Clostridium perfringens* or *Salmonella* colonies were recovered. Cooking loss on Day 0 was 29.12%–36.53%; water activity, color, and texture on Day 18 were similar between protocols. Because manufacturing was not independently replicated and conventional runs preceded modified runs, protocol effects could not be separated from run/order effects.

1. INTRODUCTION

Meat and meat-based products are important sources of protein, essential amino acids, and micronutrients. However, their high moisture content, near-neutral pH, and nutrient-rich composition favor microbial growth and biochemical deterioration during storage [1]. Meatballs are particularly susceptible to spoilage because comminution disrupts muscle structure and increases the exposure of lipids and proteins to microbial and oxidative changes.

Quality deterioration in meat products is commonly associated with microbial spoilage, lipid oxidation, protein denaturation, and off-odor development. Storage temperature and packaging conditions influence microbial growth in meatball products [2]. Vacuum and modified-atmosphere packaging are widely used to suppress microbial growth and extend refrigerated storage, although their effectiveness depends on processing and storage conditions [3].

Several preservation strategies have been investigated for refrigerated meat products, including natural antioxidants, bioactive packaging materials, and temperature-controlled storage systems [4,5]. Active packaging technologies have also been developed to improve storage stability [6]. However, these approaches may be less practical for small-scale and community-based processors because they can require additional materials, equipment, or process control. Simple, low-cost hygienic modifications may therefore provide a more feasible alternative for small-scale operations.

For small-scale meatball production, the present study therefore focused on practical hygienic modifications that could be incorporated into routine processing [2,5]. Attention to hygiene at these stages may help limit contamination without requiring sophisticated preservation technologies. The present descriptive study compared the microbiological and quality characteristics of chicken and beef meatballs produced using conventional and modified hygienic protocols during refrigerated storage at 4°C. The modified protocol comprised enhanced sanitation and personnel hygiene, crushed ice during mixing, and a cold-water cooling step after vacuum packaging.

2. MATERIALS AND METHODS

2.1. Meatball Formulation and Processing Design

Chicken and beef meatballs were prepared using commercial formulations from a small-scale meat-processing facility in southern Thailand. Chicken meatballs contained chicken meat (90%), wheat flour (6%), and seasoning ingredients (4%), whereas beef meatballs

*Corresponding Author
Atchara Nuphet Phoem, Bachelor of Science Program in Biotechnology, Faculty of Science and Technology, Songkhla Rajabhat University, Songkhla, Thailand. E-mail: atchara.ph @ skru.ac.th

contained beef meat (80%), wheat flour (14%), and seasoning ingredients (6%). Some seasoning ingredients were not disclosed because of commercial confidentiality; therefore, their possible effects on microbiological, physicochemical, and textural properties could not be excluded. Because the formulations also differed in ingredient proportions, comparisons between meat types were interpreted cautiously and were not attributed solely to meat type.

Two processing protocols were evaluated: the conventional protocol routinely used by the facility and a modified hygienic protocol developed for this study. For each meat type, one raw-material lot was divided into two portions, one for each protocol. All products were manufactured on the same day, with the conventional-protocol run conducted before the corresponding modified-protocol run. Each meat-type-protocol combination was manufactured once, and the processing order was not randomized. Thus, no independent manufacturing replication was included, and processing-protocol effects could not be separated from production-run or processing-order effects.

After completion of each conventional-protocol run and before the corresponding modified-protocol run, food-contact surfaces, equipment, and the processing area were cleaned and sanitized. The working solution was prepared by mixing 3.33 ml of a commercial product labeled as 6% sodium hypochlorite with 996.67 ml of water to obtain 1 l, corresponding to a nominal final concentration of approximately 0.02%. A contact time of 20 minutes was used. Available chlorine in the prepared solution was not independently measured.

Personnel wore hair coverings and disposable gloves: Blue gloves were used for raw-product handling, and white gloves were used for cooked-product handling. Before moving from raw to cooked handling, personnel removed the blue gloves, washed their hands with antimicrobial liquid soap and water, dried them using an air dryer, applied 70% alcohol, and donned new white gloves. Raw and cooked products were handled separately. Environmental microbiological monitoring was not conducted.

For the conventional protocol, trimmed meat was washed, minced, mixed with the formulation ingredients for 15 minutes, and shaped into meatballs. The meatballs were pre-heated at 55°C for 10 minutes and cooked in boiling water for 5 minutes. Core temperature was measured in 10 representative meatballs using a calibrated digital probe thermometer; all measured meatballs reached 72°C before transfer to a water bath at 65°C for 30 minutes. The products were then drained and vacuum-packaged in polyethylene bags.

For the modified hygienic protocol, crushed ice obtained from a commercial ice manufacturer was added during the 15-minute mixing step. The amount of ice was not standardized or recorded, batter temperatures before and after ice addition were not measured, and the microbiological quality of the ice was not tested. Shaping, pre-heating, boiling, core-temperature measurement, and the 65°C water-bath step were the same as for the conventional protocol.

After vacuum packaging, modified-protocol samples were immersed for 10 minutes in cold water at approximately 10°C. Sufficient water was used to submerge the packages, but the water volume and product-to-water ratio were not standardized or recorded. Product core temperature immediately after cooling was not measured, and complete cooling profiles and the time required to reach ≤4°C were not determined. Vacuum level and package residual oxygen were not measured for either protocol. Samples from both protocols were then stored at 4°C.

Samples were stored for 24 days, with microbiological analyses on Days 0, 6, 12, 18, and 24. Cooking loss was determined during initial production on Day 0, whereas water activity, color, and texture were evaluated on Day 18. Observations at different storage times originated from one production run for each meat type–protocol combination and were not independent batch-level replicates. Vacuum packaging and refrigerated storage were common to both protocols. The modified protocol therefore differed from the conventional protocol only in the combined application of enhanced sanitation and personnel hygiene, crushed ice during mixing, and 10-minute cold-water cooling. Separate treatment arms were not included to evaluate the individual contribution of these components.

2.2. Physicochemical and Texture Analyses

Texture profile analysis (TPA) was performed after 18 days of refrigerated storage. Samples were heated to an internal temperature of 72°C before analysis using a CT3 Texture Analyzer (Brookfield Engineering Laboratories, Middleboro, MA) fitted with a cylindrical probe at a crosshead speed of 2 mm/s. Hardness, springiness, cohesiveness, and chewiness were determined according to Beriain *et al*. [7].

Cooking loss was determined in a separate Day-0 test, rather than from mass loss across the full manufacturing sequence described in Section 2.1, using the procedure of Yildiz-Turp [8], with modifications. For each determination, 10–20 uncooked meatballs were weighed collectively, boiled until cooked, immersed in chilled water for 10 minutes, drained, air-dried to remove visible surface water, and reweighed. Cooking loss was calculated as follows:

$$\text{Cooking loss (\%)} = [(\text{Initial raw weight} - \text{Final cooked weight}) / \text{Initial raw weight}] \times 100$$

Water activity (a_w) was determined on Day 18 of refrigerated storage using a calibrated AquaLab 4TE water activity meter (Decagon Devices, Inc., Pullman, WA) following the manufacturer's measurement procedure [9].

Color was measured on Day 18 using a ColorFlex EZ colourimeter (HunterLab, Reston, VA) following the manufacturer's operating procedure [10]. Results were expressed as CIELAB L*, a*, and b* values, consistent with the color parameters described by Beriain *et al.* [7].

2.3. Microbiological Analyses

Microbiological analyses were performed on Days 0, 6, 12, 18, and 24 during refrigerated storage at 4°C using laboratory procedures modified from organism-specific Food and Drug Administration (FDA) Bacteriological Analytical Manual (BAM) methods. Because the procedures differed from the reference methods in plating format, incubation time, and confirmatory testing, they are described as laboratory-modified procedures.

For each analysis, 25 g of meatball sample was aseptically homogenized with 225 ml of sterile Butterfield's phosphate-buffered diluent to obtain a 10^{-1} homogenate. Serial 10-fold dilutions were prepared as required. Three technical replicate plates were prepared from the same homogenized sample and corresponding production run. Colony counts were recorded separately and were not pooled.

Total viable count (TVC) was determined using a pour-plate procedure modified from the FDA BAM aerobic plate count method [11]. A 1-ml aliquot of the selected dilution was transferred to a sterile Petri dish, mixed with molten Plate Count Agar, and incubated at 35°C for 24–48 hours before enumeration.

Presumptive *Staphylococcus aureus* counts were determined using a laboratory-modified pour-plate procedure with Baird-Parker agar based on the FDA BAM method [12]. A 1-ml aliquot of the 10^{-1} homogenate was mixed with molten Baird-Parker agar and incubated at 35°C for 24 hours.

Presumptive *Bacillus cereus* counts were determined using a laboratory-modified pour-plate procedure with Mannitol Egg Yolk Polymyxin agar based on the FDA BAM method [13]. A 1-ml aliquot of the 10^{-1} homogenate was mixed with the molten selective medium and incubated at 30°C for 24 hours.

Presumptive *Clostridium perfringens* counts were determined using a laboratory-modified pour-plate procedure with Tryptose Sulphite Cycloserine (TSC) agar based on the FDA BAM method [14]. A 1-ml aliquot of the 10^{-1} homogenate was mixed with TSC agar. After solidification, an additional layer of TSC agar base was applied as an overlay, and the plates were incubated anaerobically at 35°C for 24–48 hours.

For *S. aureus*, *B. cereus*, and *C. perfringens*, colonies were classified solely according to characteristic morphology on the respective selective media. No coagulase, biochemical, immunological, completed, or molecular confirmatory tests were performed; therefore, the results were interpreted as presumptive counts.

Presumptive *Salmonella* screening was performed using a laboratory-modified enrichment and isolation procedure based on the FDA BAM method [15]. A 25-g sample was pre-enriched in 225 ml of buffered peptone water at 35°C for 24 hours. Subsequently, 0.1 ml of the culture was transferred to 10 ml of Rappaport-Vassiliadis broth and incubated at 41.5°C for 24 hours. The selective enrichment culture was streaked onto pre-poured Xylose Lysine Deoxycholate (XLD) agar and incubated at 35°C for 24 hours. Plates were examined for colonies exhibiting characteristic presumptive *Salmonella* morphology. No biochemical, serological, or molecular confirmation was performed; results were therefore reported qualitatively as presumptive detection or no presumptive colonies detected in 25 g.

All culture media were obtained from HiMedia Laboratories, India. For direct enumeration, the theoretical per-plate detection limit was 10 CFU/g (1.00 log CFU/g), based on plating 1 ml of the 10^{-1} homogenate. Using 25 colonies per plate as the minimum countable number, the lower limit of quantification (LOQ) was 250 CFU/g (2.40 log CFU/g). Each plate count was converted to CFU/g and $\log_{10}$-transformed before calculation of the mean and standard deviation from the three plate-level values. Counts between the detection limit and LOQ were reported as estimated values (E), whereas samples yielding no presumptive colonies were reported as not detected (ND).

Because laboratory-modified procedures and morphology-based identification were used, the foodborne bacterial findings were considered method-specific and presumptive. They were not interpreted as definitive species confirmation or as evidence of compliance or non-compliance with the organism-specific microbiological criteria in Thai Ministry of Public Health Notification No. 416 [16]. The FDA BAM references indicate the methods from which the laboratory procedures were modified and do not imply complete application of the BAM protocols.

For descriptive comparison of TVC changes during storage, 4 log CFU/g was used as a study-selected comparative threshold based on Bassey *et al*. [5]. This value was not considered a Thai regulatory limit, a validated microbiological acceptability criterion, or a definitive shelf-life endpoint for chicken or beef meatballs.

2.4. Statistical Analysis

For each meat type–protocol combination, data were obtained from one production run only. The three microbiological plates and triplicate physicochemical and texture measurements represented technical replicates from the corresponding production run (n = 3), not independent manufacturing replicates. Data were therefore summarized descriptively as mean ± standard deviation, and no inferential statistical comparisons or process-level effects were estimated.

Microbiological counts between the theoretical per-plate detection limit and the lower LOQ were reported as estimated values (E). ND outcomes were not assigned numerical values or included in numerical summaries. Presumptive *Salmonella* screening outcomes were reported qualitatively.

3. RESULTS AND DISCUSSION

3.1. Changes in TVC During Refrigerated Storage

Figure 1 presents the TVC of chicken and beef meatballs during refrigerated storage at 4°C. TVC increased progressively in samples from both processing protocols. Values below the lower LOQ (2.40 log CFU/g) were treated as estimated counts.

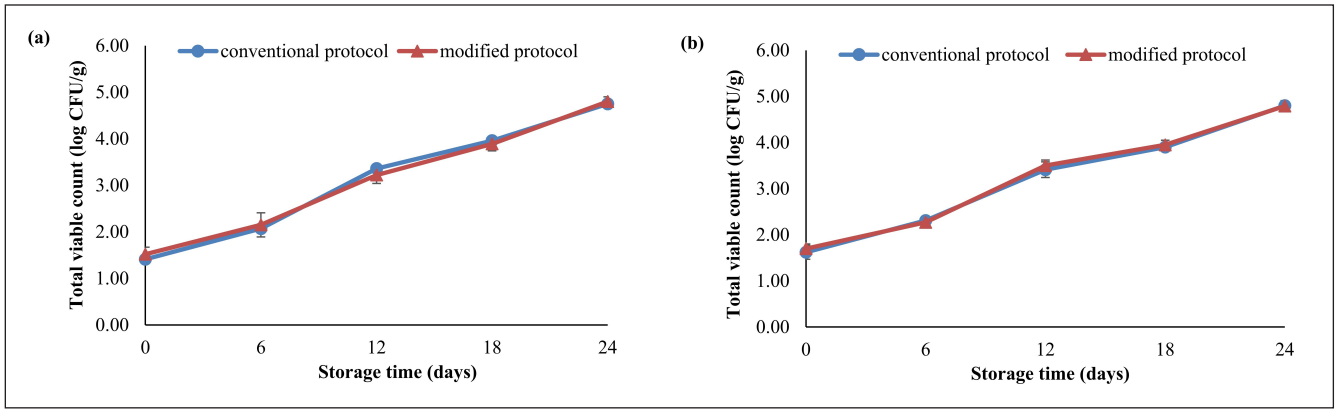

Figure 1. TVC of (a) chicken and (b) beef meatballs produced under conventional and modified hygienic protocols during refrigerated storage at 4°C for 24 days. Values are expressed as mean ± standard deviation from three technical replicate plates from one production run per meat type–protocol combination (n = 3). Data are presented descriptively; no inferential statistical comparison between protocols was performed.

For chicken meatballs (Fig. 1a), TVC increased from 1.41–1.52 log CFU/g on Day 0 to 3.89–3.96 log CFU/g on Day 18 and 4.75–4.80 log CFU/g on Day 24. Beef meatballs showed a similar numerical pattern (Fig. 1b), increasing from 1.62–1.70 log CFU/g on Day 0 to 3.90–3.95 log CFU/g on Day 18 and 4.79–4.82 log CFU/g on Day 24. Day 0 and other values below 2.40 log CFU/g were interpreted as estimated counts.

The increase in TVC during refrigerated storage was consistent with microbial growth in moisture- and nutrient-rich meat products. Similar increases during refrigerated storage have been reported in meat and meatball products [17–19]. TVC values were numerically similar between samples from the conventional- and modified-protocol runs throughout storage. Because vacuum packaging and storage at 4°C were applied to both protocols, these were not distinguishing components of the modified protocol. In addition, enhanced sanitation and personnel hygiene, crushed ice during mixing, and cold-water cooling were applied together; therefore, the contribution of each component could not be evaluated separately.

TVC remained below the study-selected comparative threshold of 4 log CFU/g through Day 18 and exceeded this value on Day 24 [5]. This threshold was used only for descriptive comparison and was not considered a Thai regulatory limit, a validated microbiological acceptability criterion, or a definitive shelf-life endpoint. Each meat type–protocol combination was manufactured once, with technical rather than independent manufacturing replication. Therefore, the observed TVC patterns describe only the production runs examined and should not be interpreted as evidence of a processing-protocol effect or as a comprehensive determination of product shelf life.

3.2. Foodborne Bacterial Counts During Refrigerated Storage

Table 1 presents the presumptive counts of selected foodborne bacteria and presumptive *Salmonella* screening outcomes during refrigerated storage at 4°C. No presumptive *S. aureus*, *B. cereus*, or *C. perfringens* colonies were detected on Days 0 and 6. From Day 12 onward, low levels of presumptive *S. aureus* and *B. cereus* were recovered, whereas no presumptive *C. perfringens* or *Salmonella* colonies were recovered using the laboratory-modified culture procedures.

On Days 12 and 18, all numerical counts were between the theoretical per-plate detection limit and the lower LOQ and were therefore reported as estimated values. On Day 24, presumptive *B. cereus* counts exceeded the LOQ in samples from both protocols, whereas presumptive *S. aureus* exceeded the LOQ only in samples from the conventional-protocol runs. Presumptive *S. aureus* counts were numerically lower in the modified-protocol runs at each sampling time, although most values were below the LOQ. Presumptive *B. cereus* counts were numerically similar between protocols and increased by Day 24.

At Day 24, all reported presumptive *S. aureus* and *B. cereus* counts were above 2.00 log CFU/g. However, organism identification was based solely on colony morphology and the culture procedures were laboratory-modified rather than complete reference methods. The results were therefore not interpreted as evidence of compliance or non-compliance with the organism-specific microbiological criteria in Thai Ministry of Public Health Notification No. 416 [16].

Each meat type–protocol combination was manufactured once, and the conventional run preceded the modified run. Consequently, the observed numerical patterns could not be separated from production-run or processing-order effects. In addition, enhanced sanitation and personnel hygiene, crushed ice during mixing, and cold-water cooling were implemented together; therefore, any numerical differences could not be attributed to an individual component of the modified protocol.

No presumptive *C. perfringens* or *Salmonella* colonies were recovered during storage. For *Salmonella*, no colonies with characteristic presumptive morphology were recovered on XLD agar following pre-enrichment and selective enrichment, and no confirmatory testing was performed. These findings therefore represent negative presumptive screening outcomes under the culture conditions applied rather than definitive evidence of organism absence. Refrigeration alone should not be regarded as a validated control for *Salmonella*, as persistence under refrigerated poultry-associated conditions has been reported [20]. The present study evaluated naturally occurring contamination only and did not include an inoculated challenge study or positive process control.

Although all 10 representative meatballs used for temperature monitoring reached a core temperature of 72°C during cooking, temperature distribution throughout the complete production runs was not evaluated. In the modified protocol, packaged meatballs were immersed in water at approximately 10°C for 10 minutes; however, product core temperature after cooling, complete cooling profiles, and the time required to reach ≤4°C were not determined. The amount and microbiological quality of crushed ice, cooling-water volume,

Table 1. Presumptive counts of selected foodborne bacteria and presumptive *Salmonella* screening outcomes in chicken and beef meatballs produced using conventional and modified hygienic protocols during refrigerated storage at 4°C.

Product	Bacterium	Day 12		Day 18		Day 24	
		Conventional	Modified	Conventional	Modified	Conventional	Modified
Chicken	*S. aureus*	1.73 ± 0.05[E]	1.20 ± 0.17[E]	1.78 ± 0.00[E]	1.10 ± 0.17[E]	2.72 ± 0.00	2.25 ± 0.01[E]
	B. cereus	1.63 ± 0.06[E]	1.52 ± 0.07[E]	1.59 ± 0.11[E]	1.69 ± 0.09[E]	2.91 ± 0.00	2.87 ± 0.00
	C. perfringens	ND	ND	ND	ND	ND	ND
	Salmonella spp.	ND	ND	ND	ND	ND	ND
Beef	*S. aureus*	1.82 ± 0.04[E]	1.20 ± 0.17[E]	1.85 ± 0.00[E]	1.20 ± 0.17[E]	2.79 ± 0.00	2.28 ± 0.02[E]
	B. cereus	1.60 ± 0.00[E]	1.52 ± 0.07[E]	1.63 ± 0.06[E]	1.56 ± 0.07[E]	2.86 ± 0.00	2.80 ± 0.00
	C. perfringens	ND	ND	ND	ND	ND	ND
	Salmonella spp.	ND	ND	ND	ND	ND	ND

Values are mean ± standard deviation (log CFU/g) from three technical replicate plates from one production run per meat type-protocol combination ($n = 3$). E, estimated count between the detection limit (1.00 log CFU/g) and LOQ (2.40 log CFU/g); ND, no presumptive colonies detected. *Salmonella* spp. results were qualitative. No confirmatory testing or inferential statistical analysis was performed.

Table 2. Cooking loss of chicken and beef meatballs measured during initial production on Day 0, and water activity and color measured after 18 days of refrigerated storage at 4°C.

Parameter	Chicken (conventional)	Chicken (modified)	Beef (conventional)	Beef (modified)
Cooking loss (%)	29.12 ± 1.45	29.65 ± 1.32	36.21 ± 1.24	36.53 ± 1.35
Water activity (a_w)	0.96 ± 0.02	0.95 ± 0.01	0.96 ± 0.01	0.97 ± 0.02
L* (lightness)	62.30 ± 1.74	64.15 ± 1.25	72.37 ± 1.20	73.45 ± 1.25
a* (redness)	1.10 ± 0.05	1.12 ± 0.04	1.65 ± 0.03	1.60 ± 0.05
b* (yellowness)	25.14 ± 1.10	26.42 ± 1.32	17.86 ± 1.37	16.55 ± 1.18

Cooking loss was measured on Day 0; water activity and color were measured on Day 18. Values are mean ± standard deviation from three technical measurements from one production run per meat type–protocol combination ($n = 3$). Data are presented descriptively; no inferential statistical analysis was performed.

product-to-water ratio, batter temperatures, vacuum level, and residual package oxygen were also not measured or standardized.

Microbial outcomes in meat systems can vary with initial contamination, packaging conditions, and antimicrobial or decontamination treatments [21–24], while storage time and temperature are particularly important for anaerobic spore-forming hazards in cooked meat products [25]. Accordingly, the present findings apply only to the production runs examined and do not establish validated lethality, cooling or packaging control, definitive pathogen absence, or a processing-protocol effect.

3.3. Physicochemical Properties of Chicken and Beef Meatballs

Table 2 presents cooking loss measured during initial production on Day 0 and water activity and color measured after 18 days of refrigerated storage at 4°C. As each meat type–protocol combination was represented by one production run, the physicochemical data are reported descriptively.

Cooking loss ranged from 29.12% to 36.53%, with values of 29.12%–29.65% for chicken meatballs and 36.21%–36.53% for beef meatballs. These values represented mass loss from the separate Day-0 cooking-loss determination and did not represent cumulative mass loss through the manufacturing sequence, storage, or reheating. Within each formulation, cooking loss was numerically similar between the conventional- and modified-protocol runs. The numerically higher values for beef meatballs may be related to differences in formulation and moisture retention. Previous studies have shown that formulation ingredients can influence water-holding capacity and cooking loss in beef and chicken meatballs [26,27]. However, direct comparison between meat types should be interpreted cautiously because the chicken and beef formulations differed in meat, flour, and seasoning proportions.

Water activity (a_w) ranged from 0.95 to 0.97 and was numerically similar among samples. Comparable physicochemical characteristics have been reported in refrigerated chicken and poultry meatballs containing different formulation ingredients [18,19]. Because the measurements were obtained as technical replicates from one production run for each meat type–protocol combination, no process-level effect on water activity can be inferred.

Beef meatballs showed numerically higher L* and a* values, whereas chicken meatballs showed numerically higher b* values. Within each formulation, color values were numerically similar between the conventional- and modified-protocol runs. Differences in pigment content, fat composition, moisture distribution, and formulation ingredients may contribute to color variation in processed meat products [17,21]. Because the formulations differed and some seasoning ingredients were not disclosed, the observed differences between chicken and beef meatballs cannot be attributed solely to meat type. Overall, these physicochemical findings describe only the production runs examined and do not establish a general processing-protocol effect.

Table 3. TPA of chicken meatballs produced using conventional and modified hygienic protocols after 18 days of refrigerated storage at 4°C.

Parameter	Conventional protocol	Modified protocol
Hardness (g)	5,378.21 ± 412.54	5,369.20 ± 347.86
Springiness	0.38 ± 0.03	0.39 ± 0.07
Cohesiveness	0.72 ± 0.03	0.75 ± 0.02
Chewiness (g)	1,532.15 ± 261.52	1,525.34 ± 250.17

Values are mean ± standard deviation from three technical measurements from one production run per protocol ($n = 3$). Data are presented descriptively; no inferential statistical analysis was performed.

Table 4. TPA of beef meatballs produced using conventional and modified hygienic protocols after 18 days of refrigerated storage at 4°C.

Parameter	Conventional protocol	Modified protocol
Hardness (g)	5,822.14 ± 535.62	5,830.20 ± 624.28
Springiness	0.37 ± 0.05	0.41 ± 0.03
Cohesiveness	0.72 ± 0.05	0.74 ± 0.05
Chewiness (g)	1,714.38 ± 265.42	1,720.15 ± 217.31

Values are mean ± standard deviation from three technical measurements from one production run per protocol ($n = 3$). Data are presented descriptively; no inferential statistical analysis was performed.

3.4. Texture Profile Analysis

Tables 3 and 4 present the texture profile results for chicken and beef meatballs after 18 days of refrigerated storage at 4°C. As each meat type-protocol combination was represented by one production run, the texture data are reported descriptively.

For chicken meatballs, hardness was 5,378.21 and 5,369.20 g in the conventional- and modified-protocol runs, respectively. Springiness ranged from 0.38 to 0.39, cohesiveness from 0.72 to 0.75, and chewiness from 1,525.34 to 1,532.15 g. For beef meatballs, hardness ranged from 5,822.14 to 5,830.20 g, springiness from 0.37 to 0.41, cohesiveness from 0.72 to 0.74, and chewiness from 1,714.38 to 1,720.15 g. Within each formulation, texture values were numerically similar between the two protocol runs.

Texture characteristics of meatballs can be influenced by formulation composition, protein-gel development, and moisture retention, and previous studies have shown that formulation ingredients can alter meatball texture [26,27]. Beef meatballs showed numerically higher hardness and chewiness than chicken meatballs; however, this pattern cannot be attributed solely to meat type because the formulations differed in meat, flour, and seasoning proportions, and some seasoning

ingredients were not disclosed. In addition, the conventional run preceded the modified run and no independent manufacturing replication was included. Therefore, the observed texture patterns describe only the production runs examined and do not establish a processing-protocol effect or a general difference between chicken and beef meatballs.

4. CONCLUSION

In the production runs examined, estimated presumptive *S. aureus* counts were numerically lower in samples from the modified-protocol runs, whereas TVC, cooking loss, water activity, color, and texture were numerically similar between protocols. Presumptive B. cereus counts were numerically similar between protocols and reached 2.80–2.91 log CFU/g on Day 24. No presumptive *C. perfringens* or *Salmonella* colonies were recovered under the culture conditions applied. However, the microbiological procedures were laboratory-modified and did not include confirmatory testing; therefore, these findings should not be interpreted as definitive pathogen absence or as evidence of regulatory compliance or non-compliance. Because each meat type-protocol combination was manufactured once and the conventional run preceded the modified run, processing-protocol effects could not be separated from production-run or processing-order effects. Independent, randomized manufacturing replicates are required before any processing-protocol effect can be established.

5. AUTHORS' CONTRIBUTIONS

All authors made substantial contributions to conception and design, acquisition of data, or analysis and interpretation of data; took part in drafting the article or revising it critically for important intellectual content; agreed to submit to the current journal; gave final approval of the version to be published; and agree to be accountable for all aspects of the work. All the authors are eligible to be author as per the International Committee of Medical Journal Editors (ICMJE) requirements/guidelines.

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7. CONFLICT OF INTEREST

The authors report no financial or any other conflicts of interest in this work.

8. ETHICAL APPROVALS

This study does not involve experiments on animals or human subjects.

9. DATA AVAILABILITY

The datasets generated during the current study are available from the corresponding author upon reasonable request.

10. USE OF ARTIFICIAL INTELLIGENCE (AI)-ASSISTED TECHNOLOGY

During revision, ChatGPT (OpenAI, GPT-5.6 Sol) was used to improve English clarity and organization in selected sections and reviewer responses. The ChatGPT image-generation feature was also used to assist with the visual design of the graphical abstract. All AI-assisted text and visual content were reviewed and revised by the authors. All scientific content, data, analyses, interpretations, and conclusions were determined and verified by the authors, who take full responsibility for the final manuscript.

11. PUBLISHER'S NOTE

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