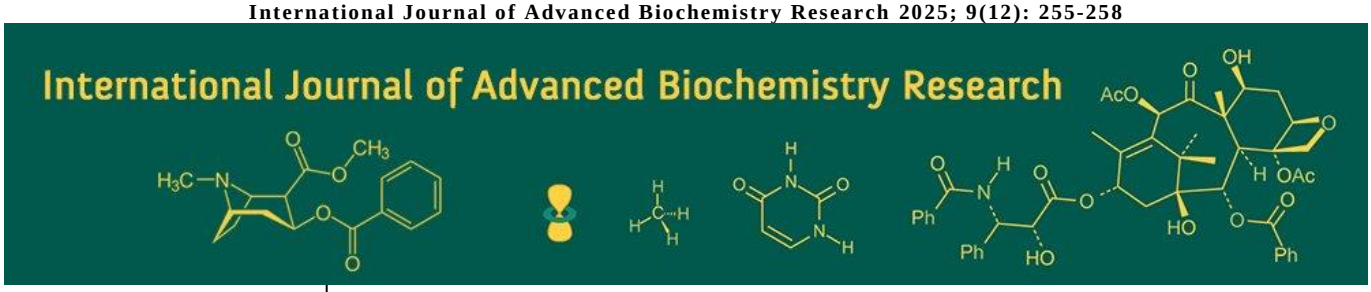

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Changes in microbial counts during fermentation of chicken sausages

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Abstract
The present study investigated the effects of *Lactobacillus plantarum* and yoghurt starter cultures on the microbial dynamics, fermentation behavior, and quality of fermented chicken sausages. Spice blends and optimized formulations were incorporated into minced chicken meat, followed by controlled fermentation at 25-28 °C. Microbiological analyses revealed a significant (p<0.05) increase in standard plate counts and lactic acid bacterial (LAB) populations throughout fermentation, with higher inoculum levels promoting faster LAB proliferation and enhanced acidification. Total plate counts increased from initial values after 24 hours, while LAB counts rose across treatments. Importantly, *Escherichia coli* and *Streptococcus* spp. were not detected at any stage following fermentation, indicating effective microbial suppression through acidification, competitive exclusion, and antimicrobial metabolites produced by LAB. The findings confirm that the use of functional starter cultures significantly enhances microbial safety, directs desirable fermentation pathways, and improves the overall microbial stability of fermented chicken sausages.

Keywords: Fermented chicken sausage, *Lactobacillus plantarum*, yoghurt starter culture, microbial dynamics, lactic acid bacteria

Introduction
Fermented sausages are widely appreciated across the world for both their traditional heritage and modern dietary value. The sequential processes of fermentation, ripening, and later cooking lead to notable modifications in their proximate and nutritional makeup, which in turn influence overall product quality, consumer preference, and associated health benefits.

Cooking adds another dimension to these changes. Methods such as boiling, steaming, grilling, and frying can significantly impact attributes like protein denaturation, water-holding capacity, lipid oxidation, and mineral concentration. Consequently, the nutritional composition and sensory qualities of fermented chicken sausages can differ depending on the cooking technique used. Understanding these variations is essential for refining processing strategies that maintain high quality while promoting consumer health.

Microbial counts including LAB, total viable counts, yeasts and molds, and potential spoilage or pathogenic organisms serve as key indicators of product quality. Over time, LAB counts may gradually increase. Regular monitoring of these microbial groups is therefore essential to assess safety and prevent quality deterioration.

In India, fermented sausages are attracting increasing research attention due to their nutritional advantages and functional prospects, especially the incorporation of probiotic cultures and bioactive compounds. These elements are associated with beneficial health effects, including improved gut microbiota and enhanced nutrient absorption. India's climatic conditions naturally support fermentation, making it an effective meat preservation method. Current studies therefore focus on product innovation enhancing sensory properties, preserving nutritional value, and retaining functional components to meet the growing demand for health-focused meat products among Indian consumers.

Review of Literature
Fermented sausages undergo a well-defined microbial succession during fermentation and

ripening that strongly influences safety, shelf life, and sensory properties. Culture-dependent and culture-independent studies consistently show a transition from a diverse raw-meat microbiota to communities dominated by lactic acid bacteria (LAB) and coagulase-negative staphylococci (CNS), with yeasts and moulds frequently colonizing casings/surfaces during ripening (Coton *et al.*, 2021; Bogdanović *et al.*, 2023) [3, 2].

Total Plate Counts (TPC)

TPC typically increase during early fermentation as fermentative bacteria multiply, then stabilize or slowly decline during drying/ripening as water activity decreases. Studies that track TPC report an initial rapid rise (days 1-3) in inoculated or naturally fermenting sausages and either a plateau or modest decline after 7-14 days depending on process conditions. In some trials inoculated sausages reach TPC values of ~10^7-10^9 CFU/g in the active fermentation phase (Adab *et al.*, 2014) [4].

Lactic acid bacteria (LAB)

LAB are the primary drivers of fermentation: their populations typically increase from low initial counts in raw meat (often <10^3-10^4 CFU/g) to dominant levels (commonly 10^6-10^9 CFU/g) within the first days of fermentation when starter cultures are used or when native LAB are active (Barbieri *et al.*, 2024) [1]. LAB proliferation lowers pH and produces metabolites (organic acids, bacteriocins) that inhibit Gram-negative and some pathogenic bacteria. The magnitude and timing of LAB growth depend on starter strain, inoculum size, temperature, and formulation (salt, nitrite, sugar).

Enterobacteriaceae and other Gram-negative groups

Enterobacteriaceae (as indicator organisms) usually decline during fermentation and ripening due to decreased pH, lowered water activity, competition from LAB, and processing hurdles (curing salts, nitrite, smoking). Many studies report progressive reductions to low or non-detectable levels by the end of ripening for properly processed products; however, poor hygiene or inadequate acidification can permit persistence. The decline is an important safety indicator because Enterobacteriaceae include spoilage species and some pathogens (Martín *et al.*, 2021) [7].

Material and Methods

Ingredients

Fresh spices (black pepper, garlic powder, paprika, curry leaves, nutmeg, and cardamom) were sourced from the local market in Bareilly, India. These were chosen for their common use in Indian meat products and their functional roles in flavor and preservation. Before use, the spices were dried in a hot-air oven and ground into fine powders to maintain uniformity and reduce microbial load. Lean chicken meat and other basic ingredients (polyphosphate, salt, vegetable oil, refined wheat flour, ice flakes, and sugar) were obtained from reliable suppliers. A commercial LAB starter culture was used to facilitate controlled fermentation.

Spice Mix Formulation

Two spice blends were prepared to study their influence on sausage sensory attributes. Mix 1 (50 g) and Mix 2 (44 g) differed slightly in the proportions of black pepper, garlic powder, and paprika. Both blends were mixed thoroughly and incorporated into sausage batters. Sensory evaluation by trained panelists assessed key attributes, and the blend with the highest acceptability was chosen for further product development (Malek *et al.*, 2025) [6].

Sausage Formulation

Minced lean chicken meat was combined with non-meat ingredients (polyphosphate, salt, ice flakes, vegetable oil, refined wheat flour, sugar) and the optimized spice blend. A freeze-dried LAB culture was added at 10^7 CFU/g. The prepared batter was filled into casings under hygienic conditions to maintain product quality.

Fermentation and Ripening

Stuffed sausages were fermented at 25-28 °C and 85-90% RH until the pH declined below 5.2. Ripening followed at 10 °C and 75-80% RH until the product reached a semi-dry texture with ~40% moisture. Temperature was monitored throughout. After ripening, the sausages were analyzed for proximate composition, microbial profile, and sensory quality.

Microbiological parameters

The microbiological parameters of Fermented Chicken sausages (In fermentation state) were evaluated following the methods described by (FSSAI, 2012). All the media used for analysis were obtained from Hi-Media® (Mumbai, India). All the microbial plates were prepared in triplicates and the count was expressed in log cfu/g.

(a) Preparation of sample and serial dilution

The stored samples at room temperature were opened and 10 g of sample was aseptically transferred into a pre sterilized mortar containing 90 ml of sterile 0.1% peptone water. The sample was homogenized for 2 minutes using a sterile pestle to achieve a uniform dispersion of sample and considered as 10-1 dilution. To make the 10-2 dilution, 1 ml of the solution was transferred into a sterile test tube containing 9 ml of sterile 0.1% peptone water and mixed thoroughly. Subsequently further dilutions were made by transferring 1 ml of the previous dilution into 9 ml of 0.1% peptone water. The appropriate dilutions inoculated into a pre-sterilised petri dish. The process of sample preparation and serial dilutions were made near to the flame inside a horizontal laminar air flow apparatus (Airstream®, ESCO class II BSC, Singapore).

(b) Total Plate Count

The Total Plate Count was determined using the pour plate method. Initially, 23.5 grams of Plate Count Agar (Hi-Media®) was dissolved in 1000 ml of double distilled water and sterilized by autoclaving at 121 °C and 15 lbs pressure for 15 minutes. After cooling to 45±2 °C, the media was ready for use with a final pH of 7.0±0.2. 1 ml aliquot of the diluted sample was then transferred to sterile disposable petri plates near a flame in a horizontal laminar airflow (Airstream®, ESCO class II BSC, Singapore), with triplicates made for each dilution. About 20-25 ml of the media was poured onto the plates, which were gently rotated to ensure even spreading before being left undisturbed to solidify. Once solidified, the plates were inverted and incubated at 35 °C for 48 hours (NSW®151 incubator, India). After incubation, colonies within the range of 30-300

were counted and the colony count was multiplied by the inverse of the dilution factor to express the results as log 10 cfu/g of the sample.

(c) Coliform count

The pour plate with overlaying method for coliform count begins by dissolving 41.5 grams of Violet Red Bile Agar (VRBA) in 1000 ml of double-distilled water, heating until fully dissolved and cooling the mixture to 45±2 °C with a final pH of 7.5±0.2. A 1 ml sample from the desired dilution is then poured into sterile petri plates under a laminar air flow cabinet and triplicates are prepared for each dilution. Approximately 20-25 ml of the cooled VRBA is added to each plate, which is gently rotated to ensure even distribution. After solidification, an additional 3-5 ml of agar is poured on top for overlaying. The plates are then inverted and incubated at 37±0.5 °C for 18-24 hours. The violet Colonies within the 30-300 range are manually counted and the result is multiplied by the inverse of the dilution factor to express the count as log 10 cfu/g of the sample.

(d) Lactic Acid Bacterial count

For the Lactobacillus count, the MRS agar (Hi-Media®) was accurately weighed 65.13g and dissolved in 1000 ml of double distilled water. The media was sterilized by autoclaving at 121 °C and 15 lbs pressure for 15 minutes. After autoclaving, the media was cooled to 45±2 °C and the pH was adjusted to 5.5±0.2. The inoculation was done as described ea. The plates were left undisturbed until the agar solidified, then inverted and incubated at 37 °C for 48 hours in an incubator (NSW®151 incubator, India). After incubation, colonies in the range of 30-300 were counted. The count was multiplied by the inverse of the dilution factor and expressed as log 10 cfu/g of sample.

Results and Discussion

Standard Plate Count (log 10 cfu/g)

The influence of *Lactobacillus plantarum* and yoghurt culture on the standard plate count (SPC) of fermented chicken sausage during fermentation at 25±2 °C is summarized in Table 5. A progressive rise in SPC was observed in all treatments as fermentation advanced. At the start (0 h), counts were relatively uniform, ranging between 5.97-6.00 log 10 cfu/g. After 8 h, treatment T_6 recorded the highest microbial load (7.80 log 10 cfu/g), whereas T_1 showed the lowest (7.24 log 10 cfu/g). By 16 h, T_6 continued to show elevated counts (8.31 log 10 cfu/g). After 24 h of fermentation, both T_6 and T_3 reached high SPC values (8.53 and 8.55 log 10 cfu/g, respectively).

Overall, significant differences (p<0.05) across treatments and time intervals indicated that microbial growth was strongly affected by the concentration of applied starter cultures. Findings agree with trends reported by Gawborisut and Muengkratok (2024) [5], who also observed increasing SPC with longer fermentation time.

Table 1: Changes in Standard Plate Counts (log 10 CFU/g) of Fermented Chicken Sausage Treated with *L. plantarum* and Yoghurt Culture at 25±2 °C

Fermentation Time (h)	T_1	T_2	T_3	T_4	T_5	T_6
0	5.97±0.08 ^{Dd}	6.00±0.08 ^{Db}	5.64±0.08 ^{Da}	5.98±0.08 ^{Dd}	5.43±0.08 ^{Da}	5.09±0.08 ^{Dc}
8	7.24±0.08 ^{Cd}	7.31±0.07 ^{Cc}	7.73±0.08 ^{Cb}	7.17±0.08 ^{Ce}	7.28±0.08 ^{Cd}	7.80±0.08 ^{Ca}
16	7.89±0.09 ^{Bc}	7.91±0.07 ^{Bc}	8.24±0.08 ^{Bb}	7.82±0.08 ^{Bd}	7.55±0.09 ^{Be}	8.31±0.08 ^{Ba}
24	8.12±0.02 ^{Ac}	8.23±0.07 ^{Ab}	8.55±0.08 ^{Aa}	7.92±0.09 ^{Ad}	8.08±0.08 ^{Ac}	8.53±0.08 ^{Aa}

n = 6, Mean ± SE, valueswithin column with different superscripts (capital letters) and within row (small letters) differ significantly (p<0.05). (T_1 = 1% *Lactobacillus plantarum*, T_2 = 2% *Lactobacillus plantarum*, T_3 = 3% *Lactobacillus plantarum*, T_4 = 1% Yoghurt Starter Culture, T_5 = Yoghurt Starter Culture and T_6 = 3% Yoghurt Starter Culture).

Lactic Acid Bacterial Count (log 10 cfu/g)

Table 2 presents the changes in lactic acid bacterial (LAB) populations in sausages fermented with varying levels of *L. plantarum* and yoghurt starter cultures. Initial LAB levels (0 h) ranged from 6.28-6.50 log 10 cfu/g, with no significant variation (p>0.05). After 8 h, LAB counts increased in all treatments, with T_6 (3% yoghurt culture) showing the highest value (7.85 log 10 cfu/g) and T_1 (1% *L. plantarum*) showing the lowest (7.49 log 10 cfu/g). At 16 h, T_6 again exhibited the highest population (8.34 log 10 cfu/g). After 24 h, T_3 and T_2 recorded the maximum LAB counts (8.60 and 8.52 log 10 cfu/g, respectively).

The observed differences (p<0.05) confirm that both starter type and concentration significantly affected LAB proliferation during fermentation. Similar fermentation-driven LAB increases were reported earlier by Stegmayer *et al.* (2023) [8].

Table 2: Changes in Lactic Acid Bacterial Counts (log 10 CFU/g) of Fermented Chicken Sausage Treated with *L. plantarum* and Yoghurt Culture at 25±2 °C

Fermentation Time (h)	T_1	T_2	T_3	T_4	T_5	T_6
0	6.28±0.08 ^{Dd}	6.34±0.09 ^{Dc}	6.50±0.01 ^{Da}	6.33±0.02 ^{Dc}	6.40±0.02 ^{Db}	6.31±0.01 ^{Dc}
8	7.49±0.01 ^{Ce}	7.57±0.01 ^{Cd}	7.75±0.09 ^{Cb}	7.56±0.05 ^{Cd}	7.69±0.01 ^{Cc}	7.85±0.08 ^{Ca}
16	8.13±0.06 ^{Bcd}	8.24±0.01 ^{Bb}	8.28±0.01 ^{Bb}	8.15±0.01 ^{Bc}	8.23±0.08 ^{Bb}	8.34±0.01 ^{Ba}
24	8.46±0.01 ^{Abc}	8.52±0.07 ^{Ab}	8.60±0.01 ^{Aa}	8.33±0.08 ^{Ad}	8.45±0.01 ^{Ac}	8.54±0.01 ^{Ab}

n = 6, Mean ± SE, valueswithin column with different superscripts (capital letters) and within row (small letters) differ significantly (p<0.05). (T_1 = 1% *Lactobacillus plantarum*, T_2 = 2% *Lactobacillus plantarum*, T_3 = 3% *Lactobacillus plantarum*, T_4 = 1% Yoghurt Starter Culture, T_5 = Yoghurt Starter Culture and T_6 = 3% Yoghurt Starter Culture).

No *Escherichia coli* or *Streptococcus* species were detected in the fermented chicken sausages after completion of the fermentation period. This absence can be attributed to the combined effects of the fermentation process and the hurdle factors present in the sausage matrix. The rapid proliferation of lactic acid bacteria (LAB) during fermentation leads to a

significant reduction in pH, typically dropping below 5.2, which creates an acidic environment that inhibits the survival and growth of acid-sensitive pathogens such as *E. coli* and many *Streptococcus* spp. Additionally, LAB produce antimicrobial substances including lactic acid, hydrogen peroxide, and bacteriocins that further suppress undesirable microorganisms. Other formulation components, such as salt, curing agents, and competition for nutrients, also contribute to microbial inhibition. The controlled fermentation temperature (25±2 °C) and reduction in water activity during ripening provide additional hurdles. Together, these factors create conditions that favor beneficial fermenting bacteria while effectively eliminating pathogenic or spoilage organisms. The absence of *E. coli* and *Streptococcus* thus indicates successful fermentation, proper starter culture activity, and effective process hygiene, confirming the microbiological safety of the final product.

Conclusion

The study clearly demonstrated that controlled fermentation using *Lactobacillus plantarum* and yoghurt starter cultures significantly influenced the microbial dynamics of fermented chicken sausages, leading to a rapid increase in beneficial lactic acid bacteria and a corresponding reduction of undesirable microorganisms. The sharp decline in pH, combined with antimicrobial metabolites produced by LAB, created an inhibitory environment that prevented the survival of pathogens, evidenced by the complete absence of *E. coli* and *Streptococcus* after fermentation. Higher starter culture concentrations accelerated LAB growth, enhanced acidification, and improved microbial stability throughout fermentation and ripening. These findings confirm that the use of functional starter cultures not only directs a desirable microbial succession but also ensures microbial safety, making them essential for producing high-quality, microbiologically stable fermented chicken sausages.

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