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Nutritional and Proximate Composition Changes in Fermented Chicken Sausage under Different Cooking Methods

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Abstract
Fermented chicken sausages (FCS) are gaining importance in India due to their nutritional significance, functional potential, and consumer acceptance. The present study evaluated the effect of different cooking methods here methods are oven cooking, smoking, and a combination of oven cooking with smoking on the proximate composition of FCS prepared using two starter cultures (*Lactobacillus plantarum* and yoghurt starter). Results revealed that cooking methods significantly influenced moisture, protein, fat, and ash contents. Oven-cooked samples showed higher moisture retention, while smoking enhanced protein, fat, and ash concentrations due to dehydration and nutrient concentration effects. Sausages fermented with yoghurt culture consistently demonstrated higher moisture and fat content compared to those with *L. plantarum*, suggesting better water-binding capacity. Overall, the findings highlight the role of both fermentation and cooking methods in determining the nutritional profile of FCS. This study supports the development of optimized processing strategies for health-oriented meat products tailored to Indian consumer preferences.

Keywords: Fermented chicken sausages, *Lactobacillus plantarum* and yoghurt starter, oven cooking, smoking

1. Introduction
Fermented sausages are valued worldwide as traditional as well as modern meat significance. The combined processes of fermentation, ripening, and subsequent cooking bring about substantial shifts in their proximate and nutritional composition, which ultimately determine product quality, consumer acceptability, and health aspects.

The cooking process introduces an additional layer of influence. Techniques such as boiling, steaming, grilling, and frying can alter proximate composition by affecting protein denaturation, water-holding capacity, lipid oxidation, and mineral concentration. As a result, the nutritional profile and eating quality of fermented chicken sausages may vary depending on the cooking method employed. Studying these changes is therefore crucial to optimize processing approaches that ensure both product quality and consumer health benefits.

Fermented sausages are gaining research interest in India due to their nutritional significance and functional potential, particularly the inclusion of probiotic microorganisms and bioactive metabolites. These components are linked to health-promoting effects, such as supporting gut microbiota and enhancing nutrient utilization. The country's climatic conditions naturally favor fermentation, making it a suitable preservation method for meat. Recent investigations therefore, emphasize product innovation, aiming to refine sensory attributes while safeguarding nutrient density and functionality, to align with the rising demand for health-oriented meat products among Indian consumers.

2. Review of Literature
During fermentation and ripening, fermented sausages undergo marked modifications in their proximate composition, largely driven by microbial activity and environmental conditions (Incili *et al.*, 2025) [4]. Among these changes, moisture reduction is the most pronounced. Initial moisture levels of approximately 60–65% may decline to nearly 30–40% in the final product (Serdaroglu *et al.*, 2022) [7]. This progressive loss of water not only concentrates

other nutrients but also improves product stability by reducing water activity, thereby limiting microbial spoilage and extending shelf life. The decrease in moisture also contributes to firmer texture and characteristic chewiness, features that are highly valued in dry and semi-dry fermented sausages (Sirini *et al.*, 2022) [8].

Protein content, generally in the range of 20–25%, remains stable in terms of absolute percentage (Yang *et al.*, 2024) [12]. However, extensive proteolysis occurs during ripening, producing peptides and free amino acids. These compounds are essential contributors to flavor development and enhance digestibility, ultimately improving the nutritional quality of the sausage. Fat, typically between 20–35%, also does not show major quantitative changes, yet lipolytic activity releases free fatty acids (Sirini *et al.*, 2022) [8]. These serve as important precursors for volatile compounds that define the aroma and taste profile of the product.

Carbohydrates, particularly fermentable sugars, decline significantly since lactic acid bacteria utilize them as substrates for energy (Wang *et al.*, 2021) [11]. This metabolism results in the formation of organic acids, mainly lactic acid, which reduces pH, improves safety, and shapes the tangy flavor associated with fermented sausages. Ash content, generally ranging between 3–5%, tends to increase slightly during ripening (Afifah *et al.*, 2023, Akwetey *et al.*, 2023) [1, 2]. This rise is not due to the addition of minerals but rather a relative concentration effect caused by moisture loss (Vignolo *et al*., 2010; Roca & Incze, 1990) [10, 6].

3. Material and Methods

3.1. Ingredients

Fresh and good-quality spices including black pepper, garlic powder, paprika, curry leaves, nutmeg, and cardamom were purchased from the local market of Bareilly, India. These spices were selected based on their traditional use in Indian meat products as well as their known sensory and preservative roles. Prior to use, all spices were subjected to drying in a hot-air oven to lower residual moisture, thereby minimizing microbial contamination and enhancing storage stability. The dried spices were then ground into fine powders to ensure uniformity and ease of blending into the sausage matrix. Other raw materials such as lean chicken meat, polyphosphate, common salt, vegetable oil, refined wheat flour, ice flakes, and sugar were procured from reliable local sources. A commercial lactic acid bacteria (LAB) starter culture was used for controlled fermentation.

3.2. Spice Mix Formulation

Two experimental spice blends were designed to evaluate their impact on the sensory profile of chicken sausages. The first blend (Mix 1) comprised black pepper (12 g), garlic powder (16 g), paprika (13 g), curry leaves (3 g), nutmeg (3 g), and cardamom (3 g), yielding a total of 50 g. The second blend (Mix 2) contained black pepper (12 g), garlic powder (13 g), paprika (10 g), curry leaves (3 g), nutmeg (3 g), and cardamom (3 g), amounting to 44 g in total. Each blend was thoroughly mixed to ensure homogeneity before being incorporated into sausage batters. Sensory evaluation was performed by a trained panel of experts specializing in meat product quality. Panelists assessed attributes including color, aroma, flavor, spiciness, saltiness, texture, mouthfeel, and overall acceptability using a structured scoring system. The formulation receiving the highest acceptability rating was selected as the optimized spice blend for further standardization and sausage preparation (Malek *et al.*,2025).

3.3. Sausage Formulation

For sausage production, lean chicken meat was minced and combined with essential non-meat ingredients such as polyphosphate, salt, ice flakes, vegetable oil, refined wheat flour, and sugar along with the optimized spice mix. The addition of polyphosphate improved water-holding capacity, while ice flakes helped maintain low batter temperature during mixing. A freeze-dried LAB starter culture was inoculated into the sausage batter at a concentration of 10^7 CFU/g to initiate fermentation. The prepared meat batter was stuffed into natural or synthetic casings under hygienic laboratory conditions to minimize contamination risks and ensure uniform product quality.

3.4. Fermentation and Ripening

The stuffed sausages were initially incubated at 25–28 °C with 85–90% relative humidity, conditions favorable for LAB activity and acidification. Fermentation was continued until the pH dropped below 5.2, ensuring microbial safety and imparting characteristic tanginess. Following fermentation, the sausages were transferred to a ripening chamber maintained at 10 °C and 75–80% relative humidity. Ripening was carried out until a semi-dry texture was obtained, corresponding to a final moisture content of approximately 40%. This step was essential for flavor development, textural firmness, and product preservation. Throughout fermentation and ripening, the internal temperature of the sausages was monitored using a calibrated digital probe thermometer. After ripening, the sausages were subjected to proximate, microbiological, and sensory analyses to evaluate their physicochemical stability and consumer acceptability.

3.5. Standardization of Cooking Procedures

Once ripening is complete, the sausages undergo standardized cooking procedures, which include oven cooking, smoking and a combination of both methods. This step is critical to ensure uniform cooking and to achieve the optimum internal temperature, making the sausages safe for consumption. Standardizing these cooking procedures ensures consistent quality across different batches, as it allows precise control over the cooking conditions. Similarly, smoking process was also standardized until attained the safe temperature and optimum flavor. After that sensory evaluation was done for all products.

Table 1: fermented sausages cooked by different methods of cooking

Treatments	Cooking
Control	Cooking without Fermentation
T1 T2	Oven cooking
T1 T2	Oven cooking + Smoking
T1 T2	Smoking

(Control= Cooking without fermentation, T1= 1% *Lactobacillus plantarum* and T2= 3% Yoghurt Starter Culture)

3.6. Proximate composition

The proximate composition of Fermented chicken sausages were analyzed using (AOAC, 2003) methods.

3.6.1. Moisture content (AOAC, 2003)

The moisture percentage of fermented chicken sausages were determined using AOAC, 2003) method.

a) For determining the moisture content, accurately weighed 2g of samples and the samples were transferred to pre weighted and dry petri dishes.

b) The samples were dried at a temperature of 100±1°C for a period of 18 hours in a hot air oven (BENCHTOP, Model/ year: BTE201D/2024).

c) After drying, the petri dishes containing samples were transferred into a desiccator and their weights were recorded after 1 hour.

Using the following equation, the moisture percentage of the samples were calculated.

$$\text{Moisture } \% = \frac{(W_2 - W_3)}{(W_2 - W_1)} \times 100$$

W1 = Empty weight of the petri dish
W2 = Total weight of fresh sample and petri dish
W3 = Total weight of dried sample and petri dish

3.5.2. Crude Protein

Crude protein content of fermented chicken sausage samples was determined using
Kjeldhal's distillation method (AOAC, 2003). The process was carried out in 3 steps namely

- Digestion, b) Distillation and c) Titration
- Digestion of samples

For digestion of samples, 0.5g of sample was accurately weighed and was transferred to a Kjeldhal's digestion flask. A pinch of catalyst (containing 0.5 parts of copper sulphate (CuSO4), 10 parts of Potassium sulphate (K2SO4) and 1 part of Ferrous sulphate (FeSO4) was added to the digestion flask. After this 10 ml of concentrated Sulphuric acid (H2SO4) was added to the digestion flask. This mixture was boiled in a tilted position until the solution became clear. After the digestion the flask was allowed to cool down and the contents was transferred into a 100 ml volumetric flask. The Kjeldhal flask was rinsed with small quantities of distilled water until all the contents were transferred to the volumetric flask. The volume was made up to 100 ml using distilled water.

- **Distillation**
  2 ml of the above solution was added to the distillation assembly followed by 5 ml of 40% NaOH. 4% Boric acid solution added with Tashiro's indicator was used for collecting the ammonia produced by distillation.
  Titration
- The quantity of Ammonia trapped inside the boric acid solution was determined by titrating it against 0.1N H2SO4
- **Calculation**

$$\% \text{ Nitrogen in the sample} = \frac{V \times V_a \times 0.0014}{W \times v} \times 100$$

Where,
V=Volume of digested samples made up to (100 ml)
Va= Volume of 0.1N H2SO4 used in titration
W= Weight of sample
V = Volume of the aliquot used for distillation.

The percentage of protein in the sample was determined by multiplying the percentage
of nitrogen in the sample with 6.25

% Protein= % Nitrogen in the sample × 6.25

3.6.3. Crude Fat

Soxhlet method (AOAC, 2003) was utilized for the estimation of crude fat of the protein bar samples using automatic fat analyser (SOCS Plus, SCS 06 RTS). Five grams of moisture free fermented chicken sausage sample was placed in extraction thimble and was secured in the sample holder which was weighed earlier and loaded in extraction unit of analyzer. Approximately 90ml of petroleum ether was loaded in sample holder for one sample. After loading the sample and solvent continuous extraction mode was chosen. Fat estimation occurred in two stages in analyser i.e. for one-hour fat extraction was done and for other 45 minutes solvent collection occurred in continuous manner. Analyzer stopped automatically once fat extraction was complete. Sample holder with extracted fat after removal of thimble were kept in hot air oven at 100 ℃ till all ether got evaporated and then in desiccator for 15 min. The sample holder was weighed for final weight and crude fat was estimated using the following equation:

$$\% \text{ Crude Fat} = \frac{W_2 - W_1}{W_3} \times 100$$

Where,
W 1 = Weight of empty flask
W 2 = Total weight of flask and fat after extraction
W 3 =Weight of sample

3.6.4. Ash Estimation (AOAC, 2003)

Percentage of ash in the Fermented chicken sausage samples were estimated using
AOAC 2003 method.

a) 2g of the sample was taken in a clean, dry and pre weighed crucible.
a) The crucibles were placed inside a muffle furnace (Khera®) at the temperature of 600°C for 8 hours.
b) The crucibles were transferred into a desiccator and they were weighed after 1 hour.

The percentage of ash in the samples were calculated using the following equation:

$$\% \text{ Total Ash} = \frac{W_1 - W_2}{W_3} \times 100$$

Where,
W 1 = Total weight of ash and crucible
W 2 = Empty weight of crucible
W 3 = Weight of sample taken

4. Results and Discussion

4.1. Proximate Analysis

The proximate analysis of fermented chicken sausages (FCS) assessed the levels of moisture, crude protein, fat, and ash across different treatments. The results indicated notable differences depending on the cooking approach applied.

Table 2: Effect of different cooking methods on Proximate analysis of Fermented chicken sausage

Cooking Method	Treatments		
	Control	T1	T2
Moisture content (%)			
Oven	47.17±0.70^{ab}	47.83±0.58^{ab}	48.30±0.47^{a}
Smoking	44.83±0.54^{Bb}	46.97±0.25^{Bb}	47.32±0.27^{Ba}
Oven Cooking and Smoking	43.83±0.70^{cc}	47.67±0.28^{Ab}	48.03±0.15^{Aa}
Crude protein (%)			
Oven	19.62±0.32^{Bb}	27.98±0.44^{Ba}	28.85±0.20^{Ba}
Smoking	20.50±0.76^{Ab}	28.75±0.31^{Aa}	29.15±0.21^{Aa}
Oven Cooking and Smoking	19.52±0.43^{Bb}	28.53±0.25^{Aa}	29.02±0.18^{Aa}
Fat content (%)			
Oven	15.24±0.09^{Bc}	17.97±0.03^{Bb}	18.16±0.04^{ca}
Smoking	17.33±0.07^{Ab}	18.68±0.03^{Aa}	18.75±0.02^{Aa}
Oven Cooking and Smoking	16.38±0.07^{Ac}	18.16±0.08^{Ab}	18.53±0.02^{Ba}
Ash content (%)			
Oven	1.22±0.19^{Bb}	2.32±0.28^{Ba}	2.55±0.20^{Ba}
Smoking	1.51±0.06^{Ab}	2.53±0.26^{Aa}	2.82±0.14^{Aa}
Oven Cooking and Smoking	1.35±0.24^{Aa}	2.72±0.22^{Bc}	2.78±0.05^{cb}

n= 6, Mean± SE, values within column with different superscripts (capital letters) and within row (small letters) differ significantly (P<0.05). (Control Cooking without fermentation, T1= 1% *Lactobacillus plantarum* and T2=3% Yoghurt Starter Culture).

4.1.1. Moisture (%)

As presented in Table 14, the moisture content of FCS varied significantly with both cooking methods and fermentation treatments. Oven-cooked samples retained the highest moisture, with T2 recording 48.30 ± 0.47%. This can be attributed to the uniform heat transfer in the oven, which supports gradual moisture retention. In contrast, smoking alone yielded the lowest values, with T1 at 46.97 ± 0.25%, likely due to enhanced evaporation during exposure to smoke. The combined method of oven cooking and smoking resulted in intermediate moisture levels. Sausages fermented with yoghurt culture generally maintained higher moisture compared to those inoculated with *Lactobacillus plantarum*. These findings are in agreement with observations reported by Susilo *et al.* (2023) [9].

4.1.2. Crude Protein (%)

The protein content of FCS, presented in Table 14, also demonstrated significant variations depending on cooking and fermentation treatments. Among the methods, smoking produced the highest protein values, with T2 reaching 29.15 ± 0.21%. Conversely, oven cooking yielded the lowest protein levels, as seen in T2 (28.85 ± 0.20%). The combination of oven and smoking produced intermediate results. The differences observed (P<0.05) suggest that smoking may be more effective at preserving protein compared to oven cooking or the combined process. Furthermore, across all treatments, T1 consistently showed slightly higher protein content than T2, aligning with the results of Susilo *et al.* (2023) [9], who reported marginally higher protein levels in sausages fermented with *Lactobacillus plantarum*.

4.1.3. Crude fat (%)

Table 1 demonstrates that the fat content of FCS is significantly impacted by different cooking methods. Smoking alone resulted in the highest fat content, with T1 at 18.68±0.03% and T2 at 18.75±0.02%. In comparison, oven cooking showed lower fat contents, with T1 at 17.97±0.03% and T2 at 18.16±0.04%. The combination of oven cooking and smoking produced intermediate fat values. Significant differences (P<0.05) suggest that smoking leads to greater fat retention, likely due to its dehydrating effect, which concentrates fat content by reducing water and minimizing fat loss. Furthermore, T2 consistently exhibited higher fat content, potentially due to the yoghurt culture's water-binding properties.

The crude fat (%) of fermented sausages with yoghurt culture showed higher fat content compared to those with *Lactobacillus plantarum*, which is consistent with the findings of Susilo *et al.*, (2023) [9].

4.1.4. Ash content%

The ash content of FCS is significantly influenced by different cooking methods, as shown in Table 14. Oven cooking resulted in ash contents of 1.22±0.19% for the control, 2.32±0.28% for T1 and 2.55±0.20% for the combined oven and smoking method slightly increased ash content, with values of 1.35±0.24% for the control, 2.72±0.22% for T1 and 2.78±0.05% for T2. Smoking alone produced the highest ash content, with 1.51±0.06% for the control, 2.53±0.26% for T1, and 2.82±0.14% for T2. The higher ash content observed in the smoking method may be due to the concentration effect resulting from moisture loss during the smoking process, which reduces water content and increases the relative proportion of mineral content in FCS. Significant differences (P<0.05) across treatments suggest that smoking generally leads to higher ash content compared to oven cooking and the combined method. Ash content (%) of fermented sausages with yoghurt culture showed higher moisture content as compared to *L. plantarum* this study show similarity with Susilo *et al.*, (2023) [9].

5. Conclusion

The study demonstrated that both fermentation cultures and cooking methods exerted a significant influence on the proximate composition of fermented chicken sausages. Smoking enhanced protein, fat, and ash concentration, whereas oven cooking preserved moisture more effectively. Yoghurt starter culture promoted higher moisture and fat retention compared to *Lactobacillus plantarum*. These findings provide insights into optimizing sausage processing techniques for enhanced quality and consumer acceptability.

6. Future Aspects

Future research may focus on:

- Exploring the incorporation of functional bioactive compounds (e.g., dietary fibers, plant extracts) to improve nutritional and health-promoting qualities.
- Investigating advanced starter cultures with probiotic potential for targeted functional benefits.
- Studying consumer preferences across diverse demographic groups to refine formulations.
- Evaluating shelf-life stability, oxidative changes, and microbiological safety under different storage and distribution conditions.
- Scaling up the optimized formulations for industrial applications to support the growing demand for healthier fermented meat products in India.

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