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Malek Aamena
MVSc Scholar, Department of Livestock Products Technology, ICAR-IVRI, Izzatnagar, Uttar Pradesh, India

Sangeeta
PhD Scholar, Department of Livestock Products Technology, LUVAS, Hisar, Haryana, India

Arman Ghasura
Business Development of Manager, Shelter Pharma, Ahmedabad, Gujarat, India

Ashutosh Khawale
MVSc scholar, Department of Livestock Products Technology, ICAR-IVRI, Izzatnagar, Uttar Pradesh, India

Nerella Venkata Pavan Kumar
MVSc Scholar, Division of Pharmacology and Toxicology, ICAR-IVRI, Izzatnagar, Uttar Pradesh, India

Avani Singh
Assistant Professor, Arawali Veterinary College, RAJUVAS, Bikaner, Rajasthan, India

Corresponding Author:
Malek Aamena
MVSc Scholar, Department of Livestock Products Technology, ICAR-IVRI, Izzatnagar, Uttar Pradesh, India

Physicochemical and biochemical responses of fermented chicken sausages to starter cultures and cooking methods

Malek Aamena, Sangeeta, Arman Ghasura, Ashutosh Khawale, Nerella Venkata Pavan Kumar and Avani Singh

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Abstract
This study evaluated physicochemical and biochemical responses of fermented chicken sausages to starter cultures and cooking methods. Sausages inoculated with *Lactobacillus plantarum* (1-3%) or yoghurt culture (1-3%) were fermented at 25-28 °C to pH ≤5.2, ripened at 10 °C, then oven-cooked, smoked, or oven-smoked. Fermentation decreased pH (~6.25→4.76-4.88), increased titratable acidity (~0.44-0.45%), and raised degree of hydrolysis (~12.5→34-38%), with higher inoculum producing greater proteolysis. During ripening (0-3 d), pH stabilized (4.70-4.85) with modest increases in acidity and hydrolysis. Fermented treatments showed lower water activity and tyrosine values than controls, indicating improved microbial stability and controlled proteolysis. Antioxidant capacity (DPPH, ABTS) was highest after smoking, especially with 3% yoghurt culture. SDS-PAGE verified loss of high-molecular-weight proteins and accumulation of peptides. Overall, starter selection and smoking acted synergistically to enhance safety, shelf life, and functional quality, providing a process framework for optimized chicken fermented sausages.

Keywords: Fermented chicken sausage, *Lactobacillus plantarum,* yoghurt starter culture, smoking, antioxidant activity, degree of hydrolysis, water activity

Introduction
Fermentation in meat systems is primarily driven by starter cultures' metabolic activity, which induces significant physicochemical and biochemical transformations in the product matrix. Lactic acid bacteria (LAB), particularly *Lactobacillus plantarum* and yogurt-based cultures, metabolize available carbohydrates to organic acids, leading to a reduction in pH. This acidification promotes protein denaturation, water-binding changes, and the inhibition of spoilage microorganisms, contributing to improved product safety and stability. Additionally, the proteolytic and lipolytic activities associated with these cultures release peptides, free amino acids and volatile compounds, which play essential roles in texture development, flavor formation and potential health-promoting functions.

The biochemical responses that occur during fermentation directly influence physicochemical parameters such as moisture content, water activity and color development. Protein breakdown during fermentation increases peptide availability, some of which exert antioxidant and antimicrobial properties, thereby improving oxidative stability and functional value of the sausage. Meanwhile, lipid oxidation and pigment stability are strongly affected by both fermentation and subsequent cooking treatments. Thermal processes such as steaming, roasting or smoking further modify protein networks, induce Maillard reaction products, develop distinct sensory attributes and influence oxidative susceptibility of lipids and pigments.

Despite the increasing consumer preference for chicken-based meat products due to their lower fat content and favorable nutritional profile, comprehensive studies examining the interplay between starter culture activity and different cooking methods in fermented chicken sausages remain limited. Understanding how microbial fermentation and thermal processing collectively modulate physicochemical and biochemical characteristics is crucial for optimizing quality, functionality and product acceptability. Therefore, the present study evaluates the effects of selected starter cultures and cooking methods on pH dynamics, color behavior, texture attributes, lipid oxidation and antioxidant properties of fermented chicken sausages.

Review of Literature

pH and Water Activity

During fermentation of meat products, starter cultures convert available carbohydrates into organic acids, primarily lactic acid, resulting in a gradual decline in pH. In fermented sausages, the initial pH generally ranges from 5.8-6.2, which progressively decreases to 4.5-5.5 depending on the rate of acid production, fermentation temperature, and microbial activity. This reduction in pH contributes to microbial safety and enhances protein coagulation, which in turn influences texture and sliceability (FSSAI, 2023). Simultaneously, water activity (aw), which initially remains high (0.95-0.98) due to moisture content in the meat batter, decreases during fermentation and subsequent drying. As fermentation progresses, aw values typically fall to 0.90-0.95, and further decline to 0.80-0.90 during maturation or drying. The combined decrease in pH and aw effectively inhibits pathogenic and spoilage microorganisms, improving both shelf stability and sensory attributes of fermented sausages (Vignolo *et al*., 2010; Roca and Incze, 1990) [21].

Antioxidant Activity

The antioxidant activity of fermented meat products is influenced by the presence of peptides, amino acids, and other bioactive compounds generated during fermentation. These compounds help mitigate oxidative stress by neutralizing free radicals, thereby contributing to product stability and potential health benefits (Jiang and Xiong, 2016). Okarini *et al*. (2019) [5, 11] reported dynamic changes in DPPH radical scavenging activity in fermented chicken sausage (bebontot), where antioxidant activity initially decreased during early fermentation but later increased as proteolysis progressed, indicating the release of antioxidant peptides. Such findings suggest that the extent and nature of proteolysis, influenced by starter cultures and fermentation duration, play a key role in determining the functional bioactivity of fermented sausages.

Degree of Hydrolysis and SDS-PAGE Analysis

The degree of hydrolysis of meat proteins during fermentation provides insight into proteolytic activity and peptide release. Wang *et al*. (2022) [22] demonstrated that Staphylococcus carnosus protease promotes hydrolysis of both myofibrillar and sarcoplasmic proteins, with a greater susceptibility observed in myofibrillar proteins. SDS-PAGE analysis is frequently used to visually verify protein degradation patterns. Ohata *et al*. (2016) [10] reported the disappearance of major protein bands such as myosin heavy chain (220 kDa) and actin (42 kDa) during fermentation, indicating significant proteolysis. The emergence and subsequent disappearance of lower molecular weight peptide bands further illustrate progressive breakdown, which is closely linked to texture softening, flavor development, and antioxidant peptide formation.

Materials and Methods

Ingredients

Fresh spices commonly used in traditional Indian meat preparations, including black pepper, garlic powder, paprika, curry leaves, nutmeg and cardamom, were sourced from the local market in Bareilly, India. The spices were selected for their flavoring properties and their known role in enhancing product preservation. Prior to use, all spices were dried in a hot-air oven to reduce residual moisture and inhibit microbial growth, after which they were finely ground to facilitate uniform incorporation into the sausage mixture. Lean chicken meat, polyphosphate, common salt, vegetable oil, refined wheat flour, ice flakes and sugar were also procured from reliable local suppliers. A commercial lactic acid bacteria (LAB) starter culture was utilized to ensure controlled fermentation.

Spice Blend Preparation

Two spice blends were formulated to assess their influence on sensory attributes of the sausage. Blend 1 consisted of black pepper (12 g), garlic powder (16 g), paprika (13 g), curry leaves (3 g), nutmeg (3 g) and cardamom (3 g), totaling 50 g. Blend 2 included black pepper (12 g), garlic powder (13 g), paprika (10 g), curry leaves (3 g), nutmeg (3 g) and cardamom (3 g), with a total weight of 44 g. Each mixture was manually blended to achieve homogeneity. Sensory evaluation was performed by a trained panel familiar with meat product profiling, who scored the samples for color, aroma, flavor, heat perception, salt balance, texture, mouthfeel and overall acceptability. The blend that received the highest overall score was selected for use in the final sausage formulation (Malek *et al*., 2025) [9].

Sausage Preparation

Lean chicken meat was ground and mixed with polyphosphate, salt, vegetable oil, refined wheat flour, sugar and ice flakes, along with the optimized spice blend. Polyphosphate was added to improve the water-binding capacity of the meat matrix, while ice flakes were used to maintain a low temperature during mixing. The freeze-dried LAB starter culture was introduced into the batter at a level of 10^7 CFU/g. The prepared mixture was filled into natural or synthetic casings under hygienic processing conditions to prevent contamination and ensure consistency.

Fermentation and Ripening

The filled sausages were placed in a controlled chamber maintained at 25-28 °C and 85-90% relative humidity to promote LAB activity. Fermentation continued until the product reached a pH of 5.2 or below, indicating sufficient acidification for safety and flavor development. After fermentation, the sausages were transferred to a ripening chamber set at 10 °C and 75-80% relative humidity until a semi-dry texture was achieved and final moisture content reached approximately 40%. Temperature was routinely monitored using a calibrated digital probe. Upon completion of ripening, physicochemical, microbiological and sensory evaluations were conducted.

Standardization of Cooking Methods

Following ripening, sausages were subjected to standardized thermal treatments, including oven cooking, smoking and a combined cooking-smoking process. Each cooking method was optimized to ensure attainment of a uniform internal temperature required for product safety. Smoking parameters were similarly adjusted to achieve consistent flavor intensity and desirable surface characteristics. Sensory evaluation was carried out after processing to determine overall product quality and consumer acceptability.

Table 1: fermented sausages cooked by different methods of cooking

Treatments	Cooking
Control	Cooking without Fermentation
T_1 **T_2**	Oven cooking
T_1 **T_2**	Oven cooking + Smoking
T_1 **T_2**	Smoking

pH Measurement

The pH of the sausage samples was determined following the method described by Trout *et al.* (1992) [18]. Approximately 10 g of the sample was homogenized with 50 mL of distilled water for 1 minute using a high-speed tissue homogenizer (IKA® ULTRA-TURRAX T 25, Germany). The pH of the resulting homogenate was measured using a digital pH meter (Hanna, HI2002-02, Italy) equipped with a combined glass electrode. Prior to each use, the instrument was calibrated using standard buffer solutions of pH 4.0 and pH 7.0 to ensure accuracy.

Titratable Acidity

Titratable acidity was determined to quantify the total acid content, primarily reflecting lactic acid accumulation during fermentation. Approximately 10 g of the sausage sample was homogenized with 90 mL of distilled water for 1-2 minutes, after which the mixture was filtered through Whatman No. 1 filter paper. A 25 mL aliquot of the filtrate was transferred to a titration flask, followed by the addition of 2-3 drops of phenolphthalein indicator. The sample was titrated against 0.1 N NaOH until a light pink color persisted for at least 30 seconds, indicating the endpoint. The titratable acidity was expressed as a percentage of lactic acid (Tyl and Sadler, 2017) using the formula:

$$\text{Titratable Acidity (\% Lactic Acid)} = \frac{(0.1\ \text{N NaOH}) \times (V_{\text{sample}} - V_{\text{blank}}) \times 75}{\text{Volume of sample (mL)}}$$

Degree of Hydrolysis

The degree of hydrolysis (DH) was assessed based on the solubility of protein in 10% (w/v) trichloroacetic acid (TCA), following the procedure of Hoyle and Merritt (1994) with minor modifications. An aliquot of 500 µL of the hydrolyzed protein solution was mixed with an equal volume of 20% (w/v) TCA, producing a final concentration of 10% TCA. The mixture was allowed to stand at room temperature for 30 minutes, followed by centrifugation at 3500 rpm for 15 minutes at refrigerated temperature (HERMLE 446K Large Volume Centrifuge, Germany). The protein content in the supernatant was quantified using the Lowry method (Lowry *et al.*, 1951) [8], with bovine serum albumin used as the standard. The degree of hydrolysis was calculated as:

DH (%) = (Protein soluble in 10% TCA / Total protein) × 100

Water Activity (aw)

Water activity (aw) of fermented chicken sausage samples was determined using a portable digital water activity meter (AquaLab 4TE, Decagon Devices, USA). Samples were cut into small uniform pieces and placed in the sample cup up to the indicated level. The cup was loaded into the measurement chamber, the lid was sealed, and the instrument was set to the measurement mode. The reading was recorded once the device stabilized and displayed the final value.

ABTS Radical Scavenging Activity

The ABTS radical scavenging activity was assessed following the method of Salami *et al.* (2009) [15]. A 7 mM ABTS stock solution was prepared and reacted with 2.45 mM potassium persulfate in equal volume to generate the $ABTS^+$ radical cation. The mixture was kept in the dark at room temperature for 16 hours. Prior to analysis, the $ABTS^+$ solution was diluted with distilled water to obtain an absorbance of 0.70±0.02 at 734 nm. For the assay, 1 mL of the working $ABTS^+$ solution was mixed with 10 µL of the sample hydrolysate, and the absorbance was measured after 20 minutes at 734 nm using a Genesys 10S UV-Vis spectrophotometer. The percentage inhibition was calculated as:

$$\text{ABTS Scavenging Activity (\%)} = \left(\frac{A_0 - A_t}{A_0}\right) \times 100$$

Where:
A_0 = absorbance at 0 min
A_t = absorbance after 20 min reaction

Tyrosine Value

Tyrosine value was estimated using a modified procedure of Strange *et al.* (1977) [16]. About 20 g of minced sausage was homogenized with 50 mL of chilled 20% TCA for 2 minutes. The homogenate was transferred to a beaker, and the container was rinsed with 50 mL of cold distilled water. The combined extract was filtered through Whatman No. 42 paper. An aliquot of 2.5 mL of filtrate was diluted with 2.5 mL distilled water and mixed with 10 mL of 0.5 N NaOH. Subsequently, 3 mL of diluted Folin-Ciocalteu reagent (1:2, v/v with water) was added. The mixture was allowed to stand in the dark for 15 minutes, and absorbance was recorded at 700 nm. Tyrosine concentration was determined using a standard curve and expressed as mg tyrosine per g of sample (Pearson, 1968) [12].

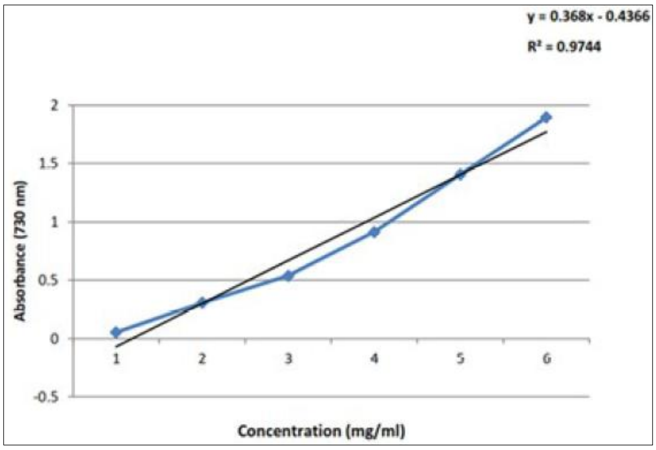

Fig 1: Standard graph of concentration of tyrosine (mg/mL) vs OD value at 730 nm

DPPH Radical Scavenging Activity

DPPH radical scavenging activity was evaluated according to Brand-Williams *et al.* (1995) [1] with minor modifications. Fresh 100 µM DPPH solution was prepared prior to each analysis. A reaction mixture was prepared by combining 1 mL of DPPH solution, 0.25 mL of 0.1 M Tris-HCl buffer (pH 7.4) and 25 µL of the sample hydrolysate. The absorbance at 517 nm was recorded immediately (A_0) and again after 20 minutes of incubation in the dark at room temperature (A_{20}). Ethanol served as the blank. The scavenging activity was calculated as:

$$\text{DPPH Scavenging Activity (\%)} = 100 - (\frac{A_{20}}{A_0} \times 100)$$

Where:
A_0 = absorbance at 0 min
A_{20} = absorbance at 20 min

SDS-PAGE Analysis
Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) was carried out to assess protein degradation patterns according to the procedure of Laemmli (1970) [7] with minor modifications.

a. Sample Preparation
Approximately 3 g of fermented sausage sample was homogenized with 9 mL of 0.05 M phosphate buffer (pH 7.4) at 11,000 rpm for 2 minutes using a tissue homogenizer (IKA® ULTRA-TURRAX T 25, Germany). The homogenate was centrifuged at 5,000 rpm for 2 minutes (REMI R8C, India), and the supernatant was collected. The centrifugation step was repeated twice to obtain a clear extract, which was used for electrophoresis.

b. Gel Preparation and Electrophoresis
A 12% resolving gel was prepared and poured between glass plates (BIO-RAD, USA), overlaid with distilled water and allowed to polymerize for 45 minutes. The water layer was removed and a 4% stacking gel was poured, followed by insertion of a comb. After polymerization, the comb was removed to form sample wells.
Protein samples were mixed with sample buffer at a 1:2 ratio (30 µL extract + 15 µL sample buffer) and heated at 95 °C for 4 minutes in a water bath (MAC MSW-275, New Delhi, India). The gel assembly was mounted in the electrophoresis unit (BIO-RAD, USA) and filled with 1× running buffer.
A 3-color prestained protein ladder (GENETIX, Cat# PG-PMT$_2$922, 10-250 kDa) was loaded (5 µL) followed by 15 µL of each sample extract. Electrophoresis was initiated at 15 mA/gel for 15 minutes, then increased to 25 mA/gel until the dye front reached near the bottom of the gel.

c. Staining and Gel Documentation
At the end of electrophoresis, gels were removed and placed in staining solution overnight. Gels were then transferred to a de-staining solution and kept until clear background bands were visible. The final gels were photographed and analyzed for band pattern interpretation.

Results and Discussion
pH
The pH of FCS was varied significantly as described in Table 2 with different cooking methods. Smoking alone resulted in the highest pH values, with 6.27±0.05 for the control, 4.84±0.04 for T_1 and 4.78±0.04 for T_2. Conversely, oven cooking produced the lowest pH values, with 4.70±0.01 for T_2, 4.76±0.01 for T_1, and 6.20±0.05 for the control. The combined oven and smoking method yielded intermediate pH values, with 6.30±0.10 for the control, 4.75±0.01 for T_1, and 4.69±0.01 for T_2.
Smoking inhibits bacterial fermentation due to dehydration, reducing acid production, while oven cooking sustains microbial activity, promoting lactic acid production. The combined method balances these effects, resulting in intermediate pH values. This aligns with Sahana *et al*., (2024) [14], who observed similar effects in dry fish.

4.1.3.5. Titratable acidity (% LA)
The titratable acidity (% LA) of fermented chicken sausages (FCS) varied significantly depending on the cooking method, as shown in Table 2. The combination of oven cooking and smoking produced the highest titratable acidity, with values of 0.26±0.02% for T_1 and 0.12±0.01% for the control. Smoking alone also resulted in high acidity, showing 0.27±0.02% for T_1 and 0.22±0.02% for T_2. In contrast, oven cooking yielded the lowest acidity values, with 0.22±0.06% for T_2, 0.21±0.02% for T_1, and 0.135±0.02% for the control.
The higher titratable acidity in smoked sausages is likely due to the introduction of acidic compounds from the smoke during the combustion process, which increases acidity and lowers pH. This effect is more pronounced in smoking compared to oven cooking or the combined method. Similar results were reported by Kudumija *et al*., (2024) [6], highlighting the impact of smoking on acid formation in FCS. The combined oven and smoking method also show elevated acidity, reflecting the combined effects of both techniques. Oven cooking results in the lowest titratable acidity, likely due to less introduction of acidic compounds and less impact on acidity compared to smoking (Huang *et al.*, 2023) [4].

4.1.3.6. Water activity (aw)
The water activity (aw) of FCS is significantly affected by different cooking methods, as shown in Table 2. Smoking alone resulted in the lowest water activity, with values of 0.92±0.01 for the control, 0.90±0.06 for T_1, and 0.91±0.05 for T_2. The combination of oven cooking and smoking also showed lower water activity, with 0.93±0.01 for the control, 0.90±0.01 for T_1, and 0.96±0.04 for T_2. Oven cooking had the highest water activity, with 0.94±0.03 for the control, 0.91±0.01 for T_1, and 0.93±0.03 for T_2. Significant differences (P<0.05) in water activity of chicken sausages indicated that smoking results in the lowest water activity, likely due to the dehydration effect of smoking, which reduces moisture content. Additionally, T_2 consistently showed higher water activity compared to T_1 and the control across all methods, reflecting the influence of the yoghurt culture.
Water activity (aw) was somewhat higher in yoghurt culture incorporated sausage over the time than *Lactobacillus plantarum* similar results was seen in study of (Susilo *et al.*, 2023) [17].

Tyrosine value (mg tyrosine per g)
The tyrosine value (mg tyrosine per g) of fermented chicken sausages (FCS) was significantly influenced by different cooking methods, as detailed in Table 16. Oven cooking resulted in the highest tyrosine levels, with values of 4.51±0.25 mg/g for the control, 3.88±0.25 mg/g for T_1, and 3.98±0.06 mg/g for T_2. The combination of oven cooking

and smoking produced slightly lower tyrosine values, with 4.53±0.24 mg/g for the control, 3.72±0.22 mg/g for T_1, and 3.78±0.05 mg/g for T_2. Smoking alone led to the lowest tyrosine levels, with 4.35±0.28 mg/g for the control, 3.85±0.17 mg/g for T_1, and 3.89±0.15 mg/g for T_2. The significant differences observed ($P<0.05$) suggest that oven cooking is the most effective method for preserving higher tyrosine levels in FCS. This may be attributed to less protein degradation during oven cooking compared to smoking, which likely contributes to the lower tyrosine values. These findings underscore the impact of cooking methods on protein stability, with oven cooking offering better retention of tyrosine content. Similar study was found in fermented sausage with *Lactobacillus plantarum* starter culture by Phupaboon and colleagues. (2022) [13].

Antioxidant Activity

Fermentation significantly enhanced the antioxidant activity of chicken sausages compared to the non-fermented control across all cooking methods. Among treatments, T_2 (3% yoghurt culture) consistently showed the highest DPPH and ABTS radical scavenging activities, followed by T_1 (1% *L. plantarum*). Smoking yielded the greatest improvement in antioxidant activity, indicating a combined effect of bioactive peptides formed during fermentation and antioxidant phenolics absorbed during smoking.

Table 2: Physico-chemical changes during fermentation of chicken sausages inoculated with *L. plantarum* and yoghurt cultures (0-24 h) (25±2 °C).

Cooking Method	Control	T_1	T_2
pH			
Oven	$6.20±0.05^{ba}$	$4.76±0.01^{bb}$	$4.70±0.01^{bc}$
Smoking	$6.27±0.05^{aba}$	$4.84±0.04^{ab}$	$4.78±0.04^{ac}$
Oven Cooking and Smoking	$6.30±0.10^{aa}$	$4.75±0.01^{bb}$	$4.69±0.01^{bb}$
Titratable acidity (% LA)			
Oven	$0.13±0.02^{ABb}$	$0.21±0.02^{ca}$	$0.22±0.06^{aa}$
Smoking	$0.14±0.02^{Ac}$	$0.27±0.02^{Ab}$	$0.22±0.02^{aa}$
Oven Cooking and Smoking	$0.12±0.01^{Bb}$	$0.25±0.02^{Ba}$	$0.26±0.03^{Ba}$
Water activity (a_v)			
Oven	$0.94±0.03^{Bb}$	$0.91±0.01^{Bc}$	$0.93±0.03^{Ba}$
Smoking	$0.92±0.01^{Bb}$	$0.90±0.06^{Aab}$	$0.91±0.05^{Aa}$
Oven Cooking and Smoking	$0.93±0.01^{Ab}$	$0.90±0.01^{cc}$	$0.92±0.04^{Aa}$
Tyrosine value (mg/g)			
Oven	$4.51±0.25^{Aa}$	$3.88±0.25^{Ac}$	$3.98±0.06^{Ab}$
Smoking	$4.35±0.28^{Bc}$	$3.85±0.17^{Ab}$	$3.89±0.15^{Ba}$
Oven Cooking and Smoking	$4.53±0.24^{Aa}$	$3.72±0.22^{Bc}$	$3.78±0.05^{cb}$

n = 6, Mean±SE. Values with different superscripts within columns (capital letters) and within rows (small letters) differ significantly ($P<0.05$).
Control = Cooking without fermentation, T_1 = 1% *Lactobacillus plantarum,* T_2 = 3% Yoghurt Starter Culture.

Table 3: Physico-chemical changes during ripening of fermented chicken sausages (0-3 days) (10±2 °C).

Parameter	Ripening (Day)	T_1	T_2	T_3	T_4	T_5	T_6
pH	0	$4.75±0.03^{Ab}$	$4.80±0.04^{Aab}$	$4.85±0.01^{Aa}$	$4.77±0.03^{Aab}$	$4.76±0.12^{Ab}$	$4.75±0.04^{Ab}$
	3	$4.70±0.09^{Aab}$	$4.77±0.05^{Aab}$	$4.73±0.09^{Aab}$	$4.79±0.03^{Aa}$	$4.73±0.05^{Aab}$	$4.71±0.03^{Ab}$
Titratable Acidity (% LA)	0	$0.44±0.08^{Ba}$	$0.45±0.06^{Ba}$	$0.44±0.09^{Ba}$	$0.44±0.08^{Ba}$	$0.44±0.09^{Ba}$	$0.45±0.08^{Ba}$
	3	$0.52±0.07^{Aa}$	$0.53±0.08^{Aa}$	$0.52±0.09^{Aa}$	$0.52±0.08^{Aa}$	$0.52±0.09^{Aa}$	$0.53±0.08^{Aa}$
Degree of Hydrolysis (%)	0	$34.35±0.40^{Bb}$	$36.17±0.26^{Aab}$	$38.06±0.87^{Ba}$	$34.22±0.34^{Bb}$	$37.15±0.99^{Ba}$	$37.12±0.32^{Ba}$
	3	$34.40±0.23^{Ac}$	$35.97±0.09^{Bbc}$	$38.35±0.76^{Aa}$	$34.40±0.76^{Ac}$	$39.46±0.14^{Aa}$	$37.61±0.07^{Ab}$
DPPH Activity (%)	0	$39.03±0.59^{Aa}$	$38.76±0.24^{Aa}$	$38.85±0.23^{Aa}$	$38.76±0.24^{Aa}$	$38.83±0.23^{Aa}$	$38.89±0.22^{Aa}$
	3	$38.20±0.36^{Aa}$	$38.25±0.35^{Aa}$	$38.34±0.33^{Aa}$	$38.24±0.35^{Aa}$	$38.32±0.33^{Aa}$	$38.05±0.02^{Ac}$

Table 4: Effect of different cooking methods on changes in the Antioxidant activity of Fermented chicken sausage

Cooking Method	Treatments		
	Control	**T_1**	**T_2**
DPPH activity (%)			
Oven	$20.27±0.25^{Cb}$	$37.44±0.13^{Ba}$	$37.70±0.06^{Ca}$
Smoking	$22.67±0.67^{Ac}$	$37.82±0.17^{Ab}$	$39.19±0.04^{Aa}$
Oven Cooking and Smoking	$22.00±0.45^{Bb}$	$37.47±0.13^{Ba}$	$38.14±0.01^{Ba}$
ABTS activity (%)			
Oven	$30.31±0.14^{Cc}$	$58.24±0.02^{Cb}$	$59.46±0.01^{Ca}$
Smoking	$34.38±0.41^{Ac}$	$59.88±0.03^{Ab}$	$63.67±0.01^{Aa}$
Oven Cooking and Smoking	$32.07±0.19^{Bc}$	$59.22±0.05^{Bb}$	$60.88±0.01^{Ba}$

n= 6, Mean±SE, means values within column with different superscripts (capital letters) and within row (small letters) differ significantly ($P<0.05$). (Control= Cooking without fermentation, T_1= 1% *Lactobacillus plantarum* and T_2= 3% Yoghurt Starter Culture).

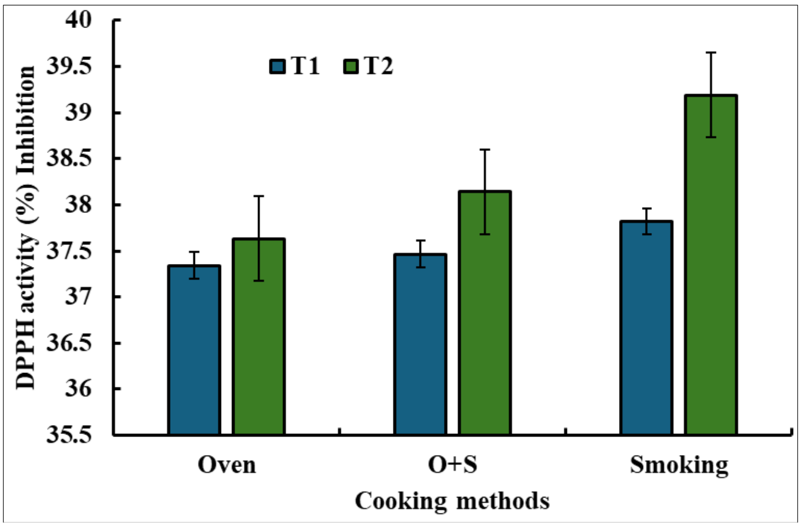

Fig 2: Effect of different cooking methods on DPPH activity (%) activity of Fermented chicken sausage

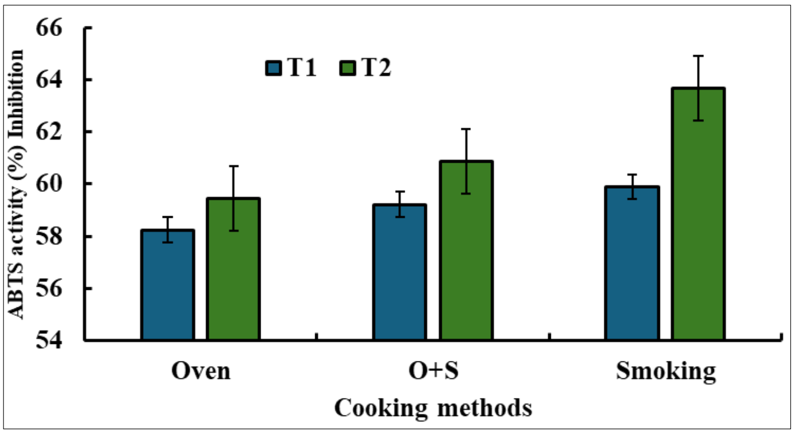

Fig 3: Effect of different cooking methods on ABTS activity (% activity of Fermented chicken sausage

Table 5: Effect of different cooking methods on changes in pH, Titratable acidity, Water activity and Tyrosin value of Fermented chicken sausage

Cooking Method	Treatments		
	Control	T_1	T_2
pH			
Oven	6.20 ± 0.05^{Ba}	4.76 ± 0.01^{Bb}	4.70 ± 0.01^{Bc}
Smoking	6.27 ± 0.05^{Aba}	4.84 ± 0.04^{Ab}	4.78 ± 0.04^{Ac}
Oven Cooking and Smoking	6.30 ± 0.10^{Aa}	4.75 ± 0.01^{Bb}	4.69 ± 0.01^{Bb}
Titratable acidity (% LA)			
Oven	0.13 ± 0.02^{ABb}	0.21 ± 0.02^{Ca}	0.22 ± 0.06^{Aa}
Smoking	0.14 ± 0.02^{Ac}	0.27 ± 0.02^{Ab}	0.22 ± 0.02^{Aa}
Oven Cooking and Smoking	0.12 ± 0.01^{Bb}	0.25 ± 0.02^{Ba}	0.26 ± 0.03^{Ba}
Water activity (a_w)			
Oven	0.94 ± 0.03^{Bb}	0.91 ± 0.01^{Bc}	0.93 ± 0.03^{Ba}
Smoking	0.92 ± 0.01^{Bb}	0.90 ± 0.06^{Aab}	0.91 ± 0.05^{Aa}
Oven Cooking and Smoking	0.93 ± 0.01^{Ab}	0.90 ± 0.01^{Cc}	0.92 ± 0.04^{Aa}
Tyrosine value (mg tyrosine per g)			
Oven	4.51 ± 0.25^{Aa}	3.88 ± 0.25^{Ac}	3.98 ± 0.06^{Ab}
Smoking	4.35 ± 0.28^{Bc}	3.85 ± 0.17^{Ab}	3.89 ± 0.15^{Ba}
Oven Cooking and Smoking	4.53 ± 0.24^{Aa}	3.72 ± 0.22^{Bc}	3.78 ± 0.05^{Cb}

n= 6, Mean±SE, means values within column with different superscripts (capital letters) and within row (small letters) differ significantly ($P<0.05$). (Control= Cooking without fermentation, T_1= 1% *Lactobacillus plantarum* and T_2= 3% Yoghurt Starter Culture).

Table 6: Effect of different cooking methods on changes in pH, Titratable acidity, Water activity and Tyrosin value of Fermented chicken sausage

Cooking Method	Treatments		
	Control	**T_1**	**T_2**
pH			
Oven	6.20±0.05^{Ba}	4.76±0.01^{Bb}	4.70±0.01^{Bc}
Smoking	6.27±0.05^{Aba}	4.84±0.04^{Ab}	4.78±0.04^{Ac}
Oven Cooking and Smoking	6.30±0.10^{Aa}	4.75±0.01^{Bb}	4.69±0.01^{Bb}
Titratable acidity (% LA)			
Oven	0.13±0.02^{ABb}	0.21±0.02^{Ca}	0.22±0.06^{Aa}
Smoking	0.14±0.02^{Ac}	0.27±0.02^{Ab}	0.22±0.02^{Aa}
Oven Cooking and Smoking	0.12±0.01^{Bb}	0.25±0.02^{Ba}	0.26±0.03^{Ba}
Water activity (a_w)			
Oven	0.94±0.03^{Bb}	0.91±0.01^{Bc}	0.93±0.03^{Ba}
Smoking	0.92±0.01^{Bb}	0.90±0.06^{Aab}	0.91±0.05^{Aa}
Oven Cooking and Smoking	0.93±0.01^{Ab}	0.90±0.01^{Cc}	0.92±0.04^{Aa}
Tyrosine value (mg tyrosine per g)			
Oven	4.51±0.25^{Aa}	3.88±0.25^{Ac}	3.98±0.06^{Ab}
Smoking	4.35±0.28^{Bc}	3.85±0.17^{Ab}	3.89±0.15^{Ba}
Oven Cooking and Smoking	4.53±0.24^{Aa}	3.72±0.22^{Bc}	3.78±0.05^{Cb}

n= 6, Mean±SE, means values within column with different superscripts (capital letters) and within row (small letters) differ significantly ($P<0.05$). (Control= Cooking without fermentation, T_1= 1% *Lactobacillus plantarum* and T_2= 3% Yoghurt Starter Culture).

SDS-PAGE Analysis

The SDS-PAGE analysis of fermented chicken sausage (FCS) revealed that the majority of protein bands appeared within the molecular weight range of 5 to 45 kDa. This indicates the presence of small to medium-sized proteins and peptides, likely resulting from fermentation and enzymatic hydrolysis during the sausage processing. The observed protein profiles suggest a significant proteolytic activity, which contributes to the breakdown of larger proteins into smaller peptides, potentially enhancing the texture and flavour development in the final product (fig.4)

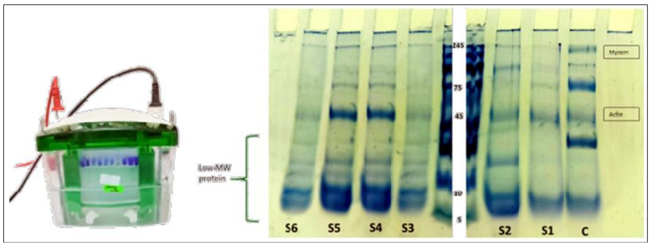

Fig 4: SDS-Page Analysis
S1 - Oven Cooking (1% *Lactobacillus plantarum*), S2 - Oven Cooking (3% Yogurt culture), S3 - Oven cooking and smoking combination (1% *Lactobacillus plantarum*), S4 - Oven cooking and smoking combination (3% Yogurt culture), S5 - Smoking (1% *Lactobacillus plantarum*), S6 - Smoking (3% Yogurt culture)

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