Research Article

Enhancing Physicochemical Properties of Chicken Sausage Through Transglutaminase and White Sorghum Incorporation

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Abstract | To diversify fillers in sausages, sorghum flour an underutilized ingredient in the food industry has been employed. However, the use of sorghum flour in sausages affects the texture, making it less compact and prone to cracking. To address this issue, the current study aimed to assess the effects of transglutaminase in white sorghum (*Sorghum bicolor*) flour on the physicochemical and nutritional characteristics of chicken sausage. A completely randomized design was employed, incorporating various levels of transglutaminase (control, 0.5%, 1%, 1.5%, and 2%) as treatments, each replicated four times. The research examined physicochemical properties, nutritional composition, and antioxidant activity. Results showed a decrease ($p < 0.05$) in cooking loss with transglutaminase addition, while higher levels led to lower ($p < 0.05$) water-holding capacity values. The optimal transglutaminase level for chicken sausages with sorghum flour was 1%. Increasing transglutaminase levels resulted in decreased ($p < 0.05$) water and fat content but increased ($p < 0.05$) ash content. The highest ($p < 0.05$) protein content was observed with 1% transglutaminase as compared to control and higher levels of transglutaminase. Transglutaminase incorporation increased sausage hardness, with the highest values at 1.5% ($p < 0.05$). Adding 2% transglutaminase significantly ($p < 0.05$) boosted antioxidant activity as compared to other levels of transglutaminase. In conclusion, incorporating 1% transglutaminase effectively enhances protein content, while a notable increase in antioxidant activity was observed at 2% transglutaminase.

Keywords | Antioxidant, Chicken sausage, Sorghum, Transglutaminase, Physicochemical properties

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INTRODUCTION

Sausages are processed foods that can be made from beef, chicken, lamb, or fish. Chicken meat is often chosen because it is more affordable and easily accessible compared to other meats. The sausage-making process involves encasing the meat in either animal intestines or artificial casings. According to National Standardization Agency of Indonesia 3820:2024 Standards (2023), sausages must have a minimum protein content of 13%. Typically, sausage production starts by grinding the meat until it is smooth, then seasoning it. The seasoned meat is then stuffed into casings and either cooked or smoked. Common ingredients used in sausage making include fillers, binding agents, flavoring agents, and preservatives.

Fillers act as stabilizers for the emulsion in sausage making and also help in forming the texture (Surfiana *et* *al*., 2024). Enhancing sausage quality has encouraged the use of diverse fillers, including underutilized resources from the food industry, such as sorghum flour. Sorghum (*Sorghum bicolor* L.) holds global significance as the fifth most important cereal crop (Khalid *et al*., 2022). It boasts high nutritional value, particularly a protein content of 10-12% (Mohammed *et al*., 2011). However, according to the findings of Permadi *et al*. (2024), the use of sorghum flour as a filler results in sausages with low cohesiveness, leading to a texture characterized by poor binding properties and a tendency to crack. To achieve a denser texture, the addition of binding agents is essential in sausage production. One effective adhesive is transglutaminase (TGase), a protein crosslinking enzyme that forms lysin-glutamine bridges by linking protein chains (Bains, 2013). Transglutaminase acts as a gel former and improves the elasticity of processed meat products, making it suitable for enhancing the characteristics of sausages (Ahhmed *et al*., 2009). Additionally, transglutaminase increases product stability during cooking, resulting in a better texture (Ismanto *et al*., 2020). This enzyme, a type of transferase, catalyzes the formation of isopeptide bonds between glutamine and lysine residues in proteins, creating stronger and more stable proteins. This improves the cohesiveness and elasticity of sausage products, thereby justifying the classification of transglutaminase as an emulsifier. These cross-links are crucial for binding proteins together, providing a cohesive texture, increasing mechanical strength, and producing a smooth, consistent texture (Duarte *et al*., 2020). Transglutaminase interacts with plant-based fillers, such as hemp protein and hemp flour, by forming inter- and intramolecular cross-links between protein particles. This enzymatic treatment enhances the technological properties of combined minced meat, resulting in a more homogeneous and compact structure. The addition of transglutaminase improves density, elasticity, final shear stress, and viscosity in the samples, while also enhancing water-binding and retention capacity. Ultimately, this contributes to better texture and reduces cooking losses in products like sausages (Zanina *et al*., 2020). Transglutaminase can induce changes in protein foods, such as alterations in size, gelation, and stability. Therefore, the texture and appearance of food can be modified using transglutaminase during processing (Vasić *et al*., 2023).

Choi *et al*. (2016) investigated the effects of transglutaminase at varying levels (0%, 0.5%, 1%, 1.5%, and 2%) on semi-dry chicken sausage. Their findings indicated that transglutaminase influenced cooking shrinkage, color, and texture, while protein content remained unchanged across treatments. Ismanto *et al*. (2020) also reported that chicken sausage formulated with transglutaminase and carrageenan exhibited a smoother texture compared to the untreated sample. While their results demonstrated a significant effect on protein content, protein levels decreased with higher transglutaminase concentrations. These findings, in conjunction with previous studies, suggest that transglutaminase and other additives have a limited impact on protein content and sausage texture. Our primary hypothesis suggests that the inclusion of transglutaminase in the chicken sausage formulation with white sorghum flour can enhance its physicochemical properties. Moreover, it is expected to affect nutritional parameters and antioxidant levels positively. Therefore, we conducted this study to assess the effects of transglutaminase and white sorghum flour on the physicochemical and nutritional characteristics of chicken sausage.

MATERIALS AND METHODS

Materials and experimental design

The chicken breast meat used in this study was obtained from a local slaughterhouse (Yogyakarta – Indonesia). The sorghum flour employed was from the KD4 variety of white sorghum, milled to an 80-mesh size. Transglutaminase, branded as WBS 500 from Wenda Ingredient (Suzhou) Co., Ltd., was used. Additionally, several other ingredients, including albumin, vegetable oil, salt, garlic powder, pepper powder, ginger powder, and nutmeg powder, were incorporated as mix-in components. This study employed a completely randomized design, with varying percentages of transglutaminase addition as the treatment variables (Control, 0.5%, 1%, 1.5%, and 2%). Each treatment was replicated four times.

Chicken sausage preparation

The ingredient proportions used for sausage preparation are detailed in Table 1 (Permadi *et al*., 2024). The chicken meat was finely chopped and ground with ice cubes, transglutaminase, and salt for one minute during the first grinding. Subsequently, albumin, vegetable oil, spices, and additional ice cubes were added and ground for one minute during the second grinding. Next, sorghum flour and additional ice were incorporated and ground for one minute during the third grinding. The thoroughly mixed dough is then placed into a sausage molding device, followed by being stuffed into sausage casings and chilled in a refrigerator at 4°C for 15 minutes. Once filled into casings, the sausages were steamed at 80°C for 10 minutes and then baked in an oven at 85°C for an additional 10 minutes. After cooling, the sausages are vacuum-packed for freezer storage. The entire sausage production process is illustrated in Figure 1.

Cooking loss measurement

Cooking loss was assessed to quantify the weight reduction during the cooking process. This evaluation involved measuring the weight of the sausage before and after cooking. The cooking loss was expressed as a percentage

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(%) using the following formula:

$$Cooking\ loss\ (\%) = \frac{weight\ before\ cooking - weight\ after\ cooking}{weight\ before\ cooking} \times 100\%$$

Table 1: Formulation of chicken sausage with white sorghum flour and transglutaminase.

Ingredients (g)	% transglutaminase				
	0	0.5	1	1.5	2
Chicken fillet	100	100	100	100	100
Transglutaminase	0	0.5	1	1.5	2
Salt	3	3	3	3	3
Albumin	10	10	10	10	10
Vegetable oil	10	10	10	10	10
Spices	12.2	12.2	12.2	12.2	12.2
Sorghum flour	10	10	10	10	10
Ice cubes	23.3	23.3	23.3	23.3	23.3

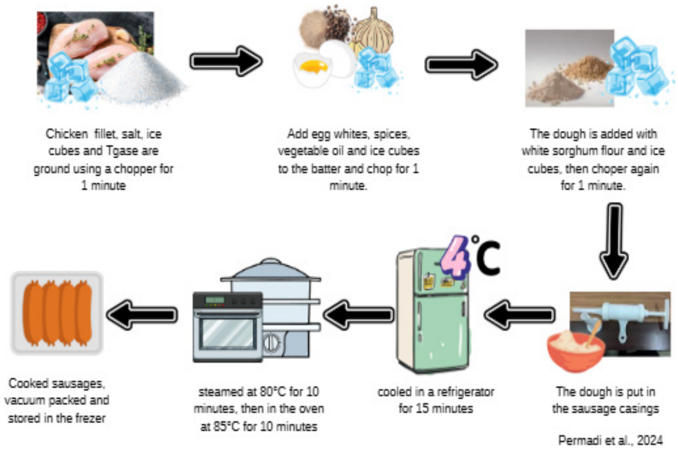

Figure 1: Chicken sausage preparation.

Water holding capacity measurement

The water holding capacity (WHC) test utilizes the compression force technique. In this procedure, a 2-gram sample is placed on filter paper and sandwiched between two plexiglass plates. A 10 kg load is then applied to the top plate for 10 minutes. After compression, the sample's weight is measured, and the WHC is calculated using the following formula:

$$WHC\ (\%) = 100 - \frac{initial\ weight\ of\ sample\ - final\ weight\ of\ sample\ \times 100}{initial\ weight\ of\ sample}$$

Moisture and nutrient content evaluation

A sample of approximately 1 gram was analyzed using the MB120 Moisture Analyzer (OHAUS Instruments, Shanghai, China). The analysis was conducted over a duration of 30 minutes. The nutrient composition, including ash content, protein, and fat, was determined following the procedure outlined in the AOAC (2005) method.

Texture measurement

The texture characteristics of sausages were examined using the TA.Xtplus texture analyzer (Stable Micro Systems, Great Britain). The texture analyzer was configured with the P36R probe model, a test speed of 5 mm/s, a force target of 5 g, a strain target of 30%, and a time target of 2 seconds for the texture profile analysis. The analysis encompassed measures of hardness, springiness, cohesiveness, gumminess, and chewiness.

Color measurement

Color properties, including lightness (L*), redness (a*), and yellowness (b*) values, were assessed using a chromameter (Konica Minolta CM-5, Japan). The minced samples of 10 g were placed in a petri dish for analysis, with each treatment unit subjected to duplicate measurements. The total color difference (ΔE*) was calculated using the following equation:

$$\Delta E^* = \sqrt{(L^*s - L^*con)^2 + (a^*s - a^*con)^2 + (b^*s - b^*con)^2}$$

Where, the L*con, a*con, and b*con values are obtained from the control sample and the L*s, a*s, and b*a values measured from the chicken sausages using sorghum and transglutaminase.

Antioxidant activity evaluation

The antioxidant activity in this study was evaluated using the 1,1-diphenyl-2-picrylhydrazyl (DPPH) assay with a spectrophotometer (Elisa Reader) at a wavelength of 517 nm. The DPPH assay involved the preparation of DPPH reagent, ascorbic acid standard, and sample solutions. A 0.2 mM DPPH solution was prepared by dissolving 3.154 mg in 40 ml of methanol in a dark tube. For the sample solution, 1 mg of the extract was dissolved in 1 ml of methanol to create a 1000 ppm solution. The ascorbic acid standard solution (100 ppm) was prepared by dissolving 0.1 mg in 1 ml of methanol, while methanol alone was used as the negative control. The test sample, standard, and negative control were then dispensed into microplate wells, each in triplicate, with 100 microliters per well, followed by adding 100 microliters of the 0.2 mM DPPH solution. After a 30-minute incubation period in the dark, the absorbance was measured at the DPPH maximum wavelength of 517 nm. The inhibition of each standard was expressed as a percentage of inhibition, as follows:

$$Inhibition\ (\%) = \frac{control\ absorbance - sample\ absorbance}{control\ absorbance} \times 100\%$$

Statistical analysis

Five TGase dosages were administered in a randomized design across four replicates for each treatment. Data analysis was performed using one-way ANOVA via SPSS 23.0 (IBM,

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Armonk, New York, USA). Means were separated using Duncan's multiple range test, with significant differences noted at p < 0.05 (Steel and Torrie, 1960).

RESULTS AND DISCUSSION

Cooking loss

Table 2 illustrates the impact of varying transglutaminase levels on the characteristics of cooking loss, water holding capacity, moisture, and nutrients in chicken sausages. The cooking loss test evaluates fluid loss during cooking, influencing the product's tenderness and nutritional content. The water loss during cooking directly influences the freshness of the meat. High-quality meat products typically exhibit lower cooking losses, associated with reduced nutrient loss during cooking (Suleman *et al.*, 2020). The introduction of transglutaminase significantly altered the cooking loss characteristics of the sausages (p<0.05). Table 2 demonstrates a reduction in cooking loss in chicken sausages following the addition of transglutaminase (p<0.05). The minimum cooking loss value was observed with the treatment of 1.5% transglutaminase level (p<0.05). These findings are consistent with previous research on frankfurters (Feng *et al.*, 2024), minced meat (Merenkova *et al.*, 2019), soy bread (Lee and Hong, 2020), beef meatballs, chicken meatballs, and turkey (Erdem *et al.*, 2020). During heating, proteins denature, leading to reduced water retention. Variations in cooking losses impact meat tenderness, with high temperatures causing structural changes, including product shrinkage. Transglutaminase can mitigate cooking losses by promoting cross-linking between protein molecules and modifying water distribution within the protein structure (Wen *et al.*, 2022). According to Irawati *et al.* (2016), changes in cooking losses are due to water loss during cooking, influenced by water-binding proteins. Increased protein water binding results in lower cooking losses, while reduced binding leads to higher water loss.

Water holding capacity

The water holding capacity (WHC) of sausages reflects product quality, indicating the meat's ability to retain moisture. WHC is influenced by various factors including composition, meat processing, and conditions such as cooking temperature and duration. thereby reducing the final product's water holding capacity (Regenstein, 2005). Our study found that adding transglutaminase at different levels significantly impacted the WHC of sausages with sorghum flour filler. The WHC values for chicken sausages with 0%, 0.5%, 1%, 1.5%, and 2% transglutaminase levels were 82.02, 81.98, 81.32, 79.91, and 79.12, respectively. Higher transglutaminase levels corresponded to lower WHC values. According to this trend, the most suitable transglutaminase level for producing chicken sausages using sorghum flour as a filler has been identified as either 0.5% or 0% (control). The elevated WHC test results were partially attributed to the incorporation of sorghum flour as a filler in chicken sausages. The presence of sorghum flour, which is rich in phenolic compounds, likely interacted with water molecules and contributed to improved moisture retention, ultimately enhancing the water holding capacity (Paes *et al.*, 2023).

A high WHC value indicates less water loss during cooking, resulting in a chewier texture. Kudryashov and Kudryashova (2023) reported that freshness and tenderness of sausages are closely influenced by their WHC. A higher WHC contributes to sausages that retain moisture and deliver an enhanced aroma. Similar to Huang and Chou (2023), a higher WHC contributes to improved texture. An increase in the proportion of sorghum-dried distillers' grains (SDDGs) is associated with an elevation in WHC. This indicates that the incorporation of sorghum in sausages effectively enhances their moisture retention properties. Meanwhile, transglutaminase significantly enhances the water holding capacity and textural properties of various protein-based foods by acting as a binding agent, facilitating protein cross-linking. This mechanism boosts water retention and reduces moisture loss during cooking. The effectiveness of transglutaminase in enhancing water-binding and textural properties depends on processing conditions. Factors like temperature, pH, and additional binding agents can influence its overall effectiveness (Lee and Hong, 2020).

Table 2: Cooking loss, water holding capacity, moisture and nutrient content of chicken sausage incorporating white sorghum flour and transglutaminase.

Treatment (% tgase)	Cooking loss	WHC	Moisture	Ash	Fat	Protein
	%		% DM			
0	11.38 ± 0.60c	82.02 ± 1.05c	63.37 ± 0.17d	2.70 ± 0.01a	6.18 ± 0.02d	18.33 ± 0.16b
0.5	10.52 ± 0.45c	81.98 ± 1.50c	62.68 ± 0.36c	2.76 ± 0.01b	6.16 ± 0.02c	19.63 ± 1.08c
1	13.03 ± 0.96d	81.32 ± 0.42bc	62.02 ± 0.29a	2.84 ± 0.02c	6.08 ± 0.02b	20.12 ± 0.79c
1.5	8.04 ± 0.44a	79.91 ± 1.07ab	61.99 ± 0.06a	2.87 ± 0.01d	5.95 ± 0.05a	18.63 ± 0.46b
2	9.34 ± 1.08b	79.12 ± 1.33a	61.67 ± 0.50a	2.88 ± 0.01d	5.89 ± 0.04a	16.41 ± 0.34a

Tgase: transglutaminase; WHC: water holding capacity; DM: dry matter. a-d values with different letters within the same column differ significantly (p<0.05).

Table 3: Texture characteristics of chicken sausage incorporating white sorghum flour and transglutaminase.

Treatment (% tgase)	Hardness (kg.f)	Springiness (%)	Cohesiveness	Gumminess (kg.f)	Chewiness (kg.f)
0	2242.60 ± 222.94^{ab}	0.878 ± 0.009^{a}	0.77 ± 0.008^{a}	1291.03 ± 87.30^{a}	1290.72 ± 79.42^{a}
0.5	2258.87 ± 307.66^{ab}	0.985 ± 0.004^{b}	0.81 ± 0.005^{bc}	1752.12 ± 38.82^{b}	1756.08 ± 17.43^{b}
1	2441.59 ± 241.00^{b}	0.993 ± 0.001^{bc}	0.82 ± 0.005^{cd}	1842.86 ± 37.67^{c}	1849.66 ± 31.45^{b}
1.5	2527.15 ± 291.19^{a}	0.990 ± 0.001^{bc}	0.83 ± 0.005^{d}	1920.55 ± 40.26^{c}	1954.22 ± 81.19^{c}
2	2278.06 ± 304.27^{b}	0.996 ± 0.005^{c}	0.80 ± 0.015^{b}	2149.59 ± 70.31^{d}	2220.28 ± 91.62^{d}

Tgase: transglutaminase, kg.f: Kilogram force. a-d superscripts with different letters within the same column differ significantly ($p<0.05$).

Moisture and nutrient content

Table 2 highlights the significant impact of adding transglutaminase on the moisture and nutrient content of sausages. According to Table 2, water content ranges from 61% to 63%, ash content ranges from 2.70% to 2.88%, fat content ranges from 5% to 6%, and protein content ranges from 16% to 20%. With increasing transglutaminase levels, water and fat content was decreased while ash content increased. The highest protein content is achieved by adding 1% transglutaminase, and the lowest with 2% transglutaminase. Based on Indonesian food standards (SNI 3820:2024), the maximum allowed contents of water, ash, and fat in sausages are 67%, 3%, and 20%, respectively, while the minimum protein content is 13%. Han *et al.* (2009) demonstrated that transglutaminase enhances protein cross-linking in the gel matrix, improving water holding capacity and reducing water content. Wen *et al.* (2022) reported that during heating, proteins denature and retain less water, leading to decreased water content in the product. Ali *et al.* (2016) noted that water absorption is influenced by starch granule structure variations, with completely altered granules reducing starch's water absorption capacity. Transglutaminase is used in sausages for its excellent binding properties, forming strong protein bonds that solidify the sausage material and help prevent organic matter loss during heating. Ash content consists of inorganic components or minerals found in food ingredients (Ismanto *et al.*, 2020).

In sausage production, meat is the main fat source, and adding low-fat fillers like sorghum reduces the overall fat content of chicken sausages. Choi *et al.* (2016) observed that sausages with 0.5% and 1% transglutaminase had lower protein content than the control (0%), while the protein content was higher with 1.5% and 2% transglutaminase. In chicken sausage, transglutaminase creates intramolecular and intermolecular cross-links between proteins through glutamine and lysine. This enzymatic modification forms a gel, stabilizing the protein network (Zhang *et al.*, 2023). Cross-linking can enhance dietary protein content by incorporating essential amino acids and facilitating protein binding in food. Fresh meat contains proteins capable of binding transglutaminase and water. Protein in food also acts as an emulsifier, contributing to a good texture (Vasić *et al.*, 2023).

Texture

Table 3 illustrates the texture characteristics of chicken sausages made with white sorghum flour and different levels of transglutaminase. Conducting texture tests is crucial for assessing the quality of chicken sausage products. Hardness is determined by the maximum force observed during the first compression, while the energy needed to separate the joints is measured as the area of positive force during both compressions (Kilic, 2003). Cohesiveness measures the sample's ability to withstand the second compression, similar to biting, compared to its behavior after the initial compression. This parameter represents the structural integrity's resistance under external forces, either compressive or tensile, and the ability to maintain its structure (Chorbadzhiev *et al.*, 2017). Chewiness assesses how well the sample returns to its original height after deformation during the first compression (Erdem *et al.*, 2020; Pagthinathan and Gunasekara, 2021). It reflects the energy required to break down food for swallowing (Ordon *et al.*, 2023). Chewiness is calculated as the product of hardness, cohesiveness, and elasticity, providing insight into the product's structural and mechanical properties during consumption and the energy needed for chewing (Chorbadzhiev *et al.*, 2017).

Transglutaminase, an enzyme known for its role in modifying protein structures, catalyzes amine incorporation, deamidation, and deamidation formation within various proteins (Erdem *et al.*, 2020). Transglutaminase promotes the creation of covalent cross-links between γ-glutamyl and ε-amino groups of lysine residues, leading to the formation of ε-(γ-glutamyl) lysine bonds. This biochemical process elongates protein chains and produces high molecular-weight polymers. The resulting bonds are approximately 20 times stronger than hydrogen bonds and hydrophobic interactions, significantly improving the mechanical and rheological properties of proteins (Wen *et al.*, 2022). However, Dong *et al.* (2020) reported that excessive addition of transglutaminase resulted in a rough surface texture in protein-rich products. Despite this, the primary industrial application of transglutaminase lies in the production of restructured meat products, where meat rich in lysine and glutamine serves as an optimal substrate for this enzyme (Erdem *et al*., 2020).

The incorporation of transglutaminase into chicken sausages increased their hardness, with the highest values observed at a concentration of 1.5% ($p < 0.05$). However, at 2% transglutaminase, hardness decreased ($p < 0.05$), suggesting that while transglutaminase enhances the protein gel structure, excessive amounts may adversely affect the product's texture (Kilic, 2003; Erdem *et al*., 2020). Additionally, cohesiveness, which measures how well a product maintains its structure under pressure, plays a significant role in texture perception. High cohesiveness can make sausages more difficult to chew, while low cohesiveness may result in brittleness, which is undesirable for consumers (Chorbadzhiev *et al*., 2017). The chewiness and springiness of sausages also increased with higher transglutaminase level ($p<0.05$), with maximum values for both parameters achieved at 2% transglutaminase. However, this increase in elasticity can adversely impact the sausage's texture, as high levels of elasticity correlate with increased hardness (Ordon *et al*., 2023). This finding is consistent with research on plant-based protein gels, where transglutaminase also improved texture parameters (Zhou *et al*., 2023). Moreover, transglutaminase is not the sole factor influencing texture; other protein sources such as sorghum and milk proteins, as well as fat and water content, can significantly impact the product's final texture (Pagthinathan and Gunasekara, 2021; Pietrasik *et al*., 2007). Additionally, Chorbadzhiev *et al*. (2017) found that large amounts of added collagen increased protein content, reduced fat, and promoted protein-protein interactions, thereby contributing to increased hardness. Studies by Bulgaru *et al*. (2022) and Mazumder *et al*. (2023) demonstrated that increasing fat and decreasing water content resulted in higher stretchability, elasticity, and chewiness.

Table 4: Color characteristics of chicken sausage incorporating white sorghum flour and transglutaminase.

Treatment (% tgase)	L*	a*	b*	ΔE
0	$64.78±0.93^{a}$	$3.10±0.14^{b}$	13.10±0.32	$0.77±0.45^{ab}$
0.5	$65.95±0.52^{ab}$	$2.98±0.05^{ab}$	13.15±0.37	$0.52±0.22^{a}$
1	$65.78±1.19^{ab}$	$2.88±0.05^{a}$	13.28±0.17	$1.09±1.10^{ab}$
1.5	$67.15±0.93^{b}$	$2.83±0.15^{a}$	13.30±0.22	$2.41±0.93^{c}$
2	$66.10±0.88^{ab}$	$3.08±1.00^{b}$	13.30±0.41	$1.88±0.86^{bc}$

Tgase: transglutaminase; *L**: lightness; *a**: redness; *b**: yellowness. a-d superscripts with different letters within the same column differ significantly ($p<0.05$).

Color

Table 4 illustrates the color characteristics of sausages with varying levels of transglutaminase. Conducting color tests is crucial for evaluating visual changes and determining the impact of transglutaminase on the final product's color attributes. Color serves as a sensory parameter that significantly influences consumer perception of product quality and market appeal.

Color plays a crucial role in product identity and consumer attraction. The CIE Lab method was employed for color testing using *L**, *a**, and *b** values. The color changes in foods containing transglutaminase are influenced by the protein content of the raw materials (Zhang *et al*., 2023). This study utilized chicken meat and white sorghum flour, which resulted in brighter colors compared to beef sausages or similar products. Statistical analysis indicated that transglutaminase concentration significantly affected all color parameters ($p<0.05$), except for yellowness (b). Sausages lacking transglutaminase exhibited the lowest L* value, indicating the darkest appearance, whereas those treated with 1.5% transglutaminase attained the highest L* value, reflecting the brightest coloration. Conversely, untreated sausages recorded the highest a* value (redness), while the 1.5% transglutaminase treatment resulted in the lowest a* value The incorporation of transglutaminase in sausages has been shown to positively correlate with an elevated b* color value when compared with no addition of transglutaminase. This indicates that as the concentration of transglutaminase increases, the sausages exhibit greater yellowness or brightness. Such an effect highlights the dual role of transglutaminase, which not only enhances the texture sausages but also contributes to their aesthetic quality by modifying their color attributes. According to Canto *et al*. (2014), the effect of transglutaminase on color changes is specific to certain types of meat, depending on the application level. The impact of transglutaminase on color variation is distinct across different types of meat, depending on the concentration applied. In sausages, transglutaminase enhances color attributes, particularly lightness and redness, which are often linked to greater consumer preference. Sausages treated with transglutaminase exhibit improved sensory qualities related to color, contributing to higher consumer acceptance levels (Cavenaghi-Altemio, 2018). The addition of transglutaminase in sausages is associated with the denaturation of pigments like globin and carotenoids, which may occur during the cross-linking process. This enzymatic activity alters the structural properties of these pigments, potentially influencing the color characteristics of the final product. Transglutaminase facilitates the formation of covalent bonds between protein molecules, resulting in a more stable and cohesive protein network. These structural modifications alter the protein matrix, which in turn influences how light is reflected or absorbed on the surface of the sausage, ultimately impacting its color properties (Santhi *et al*., 2017). The ΔE* value is utilized to detect color differences across various samples by accounting for the combined changes in *L**, *a**, and *b** values. A higher ΔE* value signifies a more significant deviation from the control group. Table 4 presents the ΔE* values, reflecting the color differences at different levels of transglutaminase application. The higher the transglutaminase addition in the sausage, the more pronounced the resulting color difference. The effect of transglutaminase is species-specific, relying on variations in enzyme concentration and myoglobin biochemistry among different meat species (Canto *et al*., 2014).

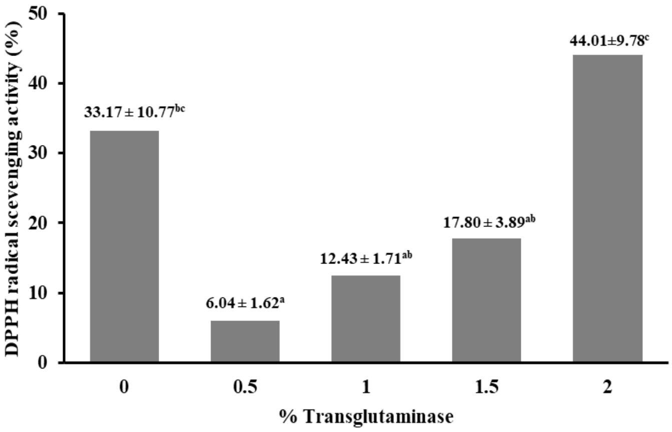

Figure 2: The antioxidant activity of chicken sausages incorporating white sorghum flour and transglutaminase. Values labelled with different letters (a–c) indicate significant differences ($p < 0.05$).

Antioxidant activity

Antioxidant activity is a critical parameter for evaluating a product's ability to neutralize free radicals, which can impact the quality and shelf life of sausages. Incorporating transglutaminase into sausage products may enhance antioxidant activity, thereby improving the nutritional value and stability of the product. The antioxidant activity of compounds in samples is typically measured using DPPH radical scavenging activity analysis, where stable free radicals in methanol undergo oxidation under strong light at a wavelength of 517 nm (Hakim *et al*., 2024). Figure 2 illustrates antioxidant of chicken sausages made with white sorghum flour and different levels of transglutaminase. The addition of transglutaminase to chicken sausages using sorghum flour as a filler showed significant results. The antioxidant values obtained were as follows: Control (33.17%), transglutaminase at 0.5% (6.04%), 1% (12.43%), 1.5% (17.8%), and 2% (44.01%). Sorghum flour plays a key role in determining the antioxidant levels in chicken sausages prepared with sorghum flour and transglutaminase. Rich in antioxidants and phenolic compounds, sorghum flour offers notable health benefits and functional advantages in food products. These bioactive elements not only enhance the nutritional profile of the sausages but also improve their structural and functional properties (Paes *et al*., 2023). Fu *et al*. (2018) reported that transglutaminase catalyzes the transfer of acyl groups and forms covalent cross-links between myosin and casein, thus enhancing protein flexibility. Moreover, the effectiveness of transglutaminase can be maximized when combined with other chemicals. For instance, adding antioxidant chemicals to gel products may lead to quality degradation, which can be mitigated by transglutaminase. Transglutaminase increases the hydrophobicity of protein structures, reduces irregular cross-links of myofibrillar proteins, improves uneven protein aggregation, and promotes the formation of irregular macroporous gels (Chang *et al*., 2023).

CONCLUSIONS

Incorporating transglutaminase into chicken sausage, using white sorghum flour as a filler enhances its nutritional value and physical properties. These enhancements are observed through reductions in cooking loss, moisture, and fat content. Integration of 0.5%-1% transglutaminase can increase the protein content in chicken sausages. Furthermore, a marked improvement in antioxidant activity is observed when the transglutaminase concentration is increased to 2%. It is important to consider alterations in color and texture separately, as these will influence consumer acceptance. The optimal levels of transglutaminase addition for white sorghum flour-based chicken sausage are 1% and 2%. However, there is a need to enhance the color and texture properties. Future research should include sensory evaluations to determine consumer acceptance, alongside industrial-scale production trials to assess the practicality and scalability of the formulation. These efforts establish a robust basis for further product development and facilitate its wider adoption in commercial applications.

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NOVELTY STATEMENT

This study presents a novel approach by incorporating transglutaminase into chicken sausage with white sorghum flour as a filler, effectively enhancing its nutritional profile and physical properties. The inclusion of 0.5%-1% transglutaminase leads to an increase in protein content, while a concentration of 2% improves antioxidant activity.

These findings expand the understanding of white sorghum flour applications in sausage production and provide a foundation for future research in functional meat products.

AUTHOR'S CONTRIBUTION

Permadi designed and supervised the experiment, prepared raw samples, performed laboratory analysis, collected the data, wrote the first draft manuscript, and revised the manuscript; Wahyono supervised the experiment, validation, wrote the first draft, and revised the manuscript; Wahyuningsih prepared raw samples and performed laboratory analysis. Saputri performed laboratory analysis, collected the data and revised manuscript; Hakim prepared raw samples and performed laboratory analysis; Firmansyah prepared raw samples, performed laboratory analysis, and collected the data; Aditya collected the data and revised the manuscript; Febrisiantosa designed the experiment and revised the manuscript; Ramadhani prepared raw samples, performed laboratory analysis, collected the data, analyzed the data and visualization; Yulviatun designed the experiment and revised the manuscript

Conflict of interest

The authors have declared no conflict of interest.

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